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Determination of Diodrast (Diodone) in Blood and Urine According to a Modification of White and Rolf's Method.

By

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Received 17 Februay 1948.

Since ELSOM, BOTT and WALKER (1937) discovered the connection between the renal blood flow and the clearance of certain iodine-containing substances, and SMITH and co-workers (1938, 1940) studied the behaviour of normal and pathological kidneys, several methods and modifications have been developed for the determination of diodrast iodine and allied compounds in blood and urine (WHITE and ROLF 1940, ALPERT 1941, FLOX, PITESKY and ALVING 1942, BAK, BRUN and RAASCHOU 1943, CORCORAN and PAGE 1943, BARCLAY and KENNEY 1945). FINKELSTEIN et al. (1941) found that para-amino-hippuric acid not only is excreted by the kidneys just like diodrast, but also is easy to determine quantitatively, but this compound is not universally available nor is it easy to prepare. It is therefore important to find a method for determining diodrast iodine which is as rapid and as accurate as the hippuric acid method.

One may, practically speaking, attribute all the iodine found by analysis of the plasma to the diodrast as plasma itself contains only about 13 gamma iodine per 100 ml (BIILMAN 1938). The analysis is carried out titrimetrically or colorimetrically after oxidation of the iodine compound to iodate and subsequent elimination of the excess of oxidizing agent. The oxidation is

Carried out with support from "Miss P. A. Braudt's Legacy".

achieved by means of potassium permanganate or bromine while different reducing agents are employed for the removal of excess oxidizing agent. In plasma it is necessary first to precipitate the proteins.

The various analytical methods published all suffer from more or less serious defects: ALPERT's (1941) shows too high values at low concentration in plasma so the least quantity analyzable was 10 micrograms, moreover it is too tedious for routine use. Blank values were found in FLOX, PITESKY and ALVING's method (1942) and in CORCORAN and PAGE's (1943). BARCLAY and KENNEY's method (1945) shows now and then blank values using tungstic acid as precipitating agent but no blank value using trichloroacetic acid, BARCLAY and KENNEY have *not* mentioned the uncertainty of the method. Bromine is used as oxidizing agent in all these methods, and it is seen that the methods all have blank values but not systematic losses. When permanganate is the oxidizing agent there is no blank value but a systematic loss, this is seen in WHITE and ROLF's method (1940) and in the modification presented by BAK, BRUN and RAASCHOU (1943). The latter also shows too high values at low concentrations so the least quantity analyzable is 8 micrograms. The "systematic losses" are unfortunate, because you can not be sure that they really are systematic.

The systematic loss when permanganate is the oxidizing agent may, in the case of plasma, be attributable to the precipitated proteins or to a direct influence of the precipitating agent on the processes that occur later in the analysis. The former explanation is supported by the circumstance that the diodrast-iodine can be recovered completely when added to a protein-free plasma filtrate, the second by the circumstance that no loss occurs in the bromine methods, even though the same precipitating agent is used for the proteins.

BAK, BRUN and RAASCHOU attribute the systematic loss in their analysis of plasma to an adsorption of diodrast during the precipitation. They find that the loss is less when tungstic acid is used as precipitating agent instead of WHITE and ROLF's trichloroacetic acid which they regard as having a reducing effect on the iodate formed later in the analysis.

Preliminary experiments with BAK, BRUN and RAASCHOU's modification have shown, however, that the systematic loss can be reduced by using smaller amounts of sulfuric acid in the potas-

sium permanganate oxidation. It is found that if one-third of the sulfuric acid is neutralized before the addition of the solium nitrite, the systematic loss falls from 8 % to 2 %. This has been further investigated.

If the systematic loss occurs after the precipitation of the proteins it must be because some of the iodate formed is reduced by some reagent added. Nitrite is used for the purpose of removing the excess potassium permanganate, and this nitrite may reduce iodate according to the equation:

$$2H^+ + 2JO_3^- + 5NO_2^- = 2J + 5NO_3^- + H_2O$$

The process has a tendency to proceed towards the right, and the more so the higher the hydrogen ion concentration.

Assuming that this is the process responsible for the systematic loss in the analysis, an investigation was carried out to see how it might be depressed, either by changing the pH or by adding oxidizing substances. To a solution containing a known amount of iodate, nitrite was added along with a certain amount of acid. After boiling for 30 minutes the excess nitrite was removed by means of urea, and the remaining iodate was titrated iodometrically. The results were as follows, the volume of the solution being 4 ml and the pH that measured after 15 minutes of boiling:

Acid added			pH	Iodate recovery
0.8	milliequivalent	H_2SO_4	1.8	0 %
0.4	»	»	3.7	3 %
1.12	»	HNO_3	<1.0	2.5 %
0.4	»	»	3.0	35 %

It will be seen 1) that nitrite in this experiment, under certain conditions, is capable of reducing iodate completely, 2) that the reduction depends on the amount of acid used and thus on the pH, and 3) that the recovery is larger when the oxidizing nitric acid is used instead of the original sulfuric acid.

If the systematic loss in the permanganate method is due to the reducing effect of nitrite according to the above equation, it should therefore be possible to cut this loss by using less sulfuric acid or by replacing this acid with nitric acid. The former modification proved effective, to be sure, but with the small amount of sulfuric acid, the ring of MnO_2 which is liable to be formed during the permanganate treatment, was difficult to remove completely and therefore affected the end-point of the titration.

Nitric acid was then used and was found to eliminate the systematic loss completely in experiments where this modification was compared with the method of BAK, BRUN and RAASCHOU with large and small amounts of diodrast iodine in the same volume of deproteinized plasma; tungstic acid being used in both instances.

Original amount of iodine.	Recovery percentage in B., B. and R.'s method	Recovery percentage in the author's modified method
45.5 micrograms	89 %	98.8 %
15.15 »	92.5 %	102.3 %

The method of BAK, BRUN and RAASCHOU gave no systematic loss when applied to aqueous solutions of diodrast, while such loss occurred when the diodrast was dissolved in plasma. It is rather difficult to explain this difference, as it has been demonstrated that the protein precipitation cannot be responsible. Analyses were therefore made of aqueous solutions of diodrast to which the precipitating agent tungstic acid, used by BAK, BRUN and RAASCHOU, was added. The loss was now found to be 8 %, or precisely the same as in plasma when tungstic acid is used to precipitate the proteins. Both losses could be reduced when one-third of the sulfuric acid used with the permanganate was neutralized before nitrite was added for removal of the excess oxidizing agent. It may be possible, therefore, that the tungstic acid has a catalytic effect on the nitrite reduction of iodate.

Description of the Analysis.

Reagents:

a) Zinc hydroxide prepared by adding 5 volume 0.45 % zinc sulfate to 1 volume N/10 sodium hydroxide.

b) 1.40—1.36 N nitric acid prepared by dilution 10: 1 of pure concentrated nitric acid (specific gravity 1.43).

c) 6 % potassium permanganate (reagent grade).

d) 1 N sodium hydroxide.

e) 7 % sodium nitrite (reagent grade).

f) A solution of 75 g of urea in 250 g of 4 N acetic acid.

g) A solution of 2 g of potassium iodide (reagent grade) in 6 ml of distilled water.

h) 1 % starch solution.

i) N/200 potassium iodate (accurate standard solution).

j) N/1000 sodium thiosulfate — to be renewed at frequent intervals by dilution of an N/200 solution which is more stable.

Carbon dioxide from a cylinder, and ice-water.

Equipment:

This includes a 2 ml Krogh-Rehberg micro titration burette, graduated in 0.01 ml divisions, 0.3 ml and 0.5 ml micro pipettes, as well as 16 × 180 mm test tubes which should be used exclusively for this work as it is then only neeessary to rinse them with water between determinations.

Procedure:

In the case of plasma, the first step is to remove the proteins.

1) Add 10 ml of freshly prepared zinc hydroxide (reagent a) to 1 or 0.5 ml of plasma (thus diluting it 11 or 21 times respectively) and boil for 5 minutes in water bath in a centrifuge tube stoppered with water-repellant cotton-wool.

Note: While originally Somogyi's zinc hydroxide was used, it was found that too much plasma supernatant was lost in the voluminous precipitate. With the method adopted the boiling gave only a very small evaporation loss, namely less than 0.5 %. Tungstic acid. as used by Bak, Brun and Raaschou, is less satisfactory.

2) Centrifuging for 10 minutes at about 3000 r. p. m.

The clear supernatant is now treated like aqueous solutions, enough being transferred to the test tubes to permit double determinations.

3) Add up to 4 ml of this supernatant to 1 ml of nitric aeid (reagent b) plus 0.3 ml of potassium permanganate (reagent c).

Note: For plasma supernatant one should use a pipette having a long fine tip when transferring to the test tubes. Otherwise the preeipitate may be stirred up, though no real harm is done by the presenee of a few flakes in the solution. It is important that the upper half of the test tube is not splashed by the potassium permanganate solution as drying out during the boiling otherwise will produee a coating which it is diffieult to remove later on by the nitrite and which will give rise to a blank value in the titration. Any coating farther down in the tube will be washed off by the eondensed water vapours. While the volume of the liquid is not so important, it should nevertheless be between 4 and 8 ml. If more than 8 ml, the aceuracy of the titration is affected, and if less than 4 ml the eoneentration of potassium permanganate will be too high. The nitric acid solution will not keep for more than a couple of months and should be tested now and then; if too weak, the end-point of the titration may become indefinite.

4) Boil for 10 to 15 minutes in a water bath.

Note: If the time of boiling is less than 10 minutes, the oxidation will be incomplete, if more than 15 minutes the above mentioned ring of MnO_2 is apt to form in the test tube. The water of the bath should be a little above the level of the solution in the tubes.

5) Add 1 ml of sodium hydroxide (reagent d).

Note: This serves not only to remove excess acid so that the above mentioned reduction of iodate does not oceur, but it also increases the volume of the solution so that the MnO_2 ring gets below the surface where it is readily removed during the subsequent boiling.

6) Remove test tubes one by one, shake carefully and then add 0.3 ml sodium nitrite (reagent e). Shake once more carefully until the remaining colour is but faintly brown.

Note: Careful shaking before and after the addition of nitrite is necessary as otherwise an excess of MnO_2 is formed.

7) Boil for 10 to 15 minutes.

Note: The influence of the boiling time was tested in an experiment on the percentage recovery at different times which showed that short boiling gives rise to blank values:

	Time of boiling		
	5 minutes	10 minutes	20 minutes
4 micrograms: Recovery.....	110 %	99 %	101 %
50 micrograms: Recovery.....	103.5 %	100 %	99.5 %

8) Remove test tubes once more and shake carefully. Then add 1 ml of reagent f and shake once more until a slight effervescence occurs.

Note: Since the reaction

$$CO(NH_2)_2 + 2\,HNO_2 = CO_2 + 2\,N_2 + 3\,H_2O$$

will not proceed quantitatively towards the right unless the solution is acidic, the acetic acid is added along with the urea.

9) Boil for 5 minutes, shake the tubes once and boil again for 2—3 minutes. Then transfer test tubes to ice-water.

Note: The total boiling time should be between 5 and 10 minutes. The cooling in ice-water is necessary if iodine is to react properly with starch at low iodine concentration. This observation (12) has been confirmed in own experiments.

10) Add to each test tube 0.2 ml of potassium iodide (reagent g) and 0.5 ml of starch solution (reagent h). CO_2 is led through by means of a capillary tube.

Note: This concentration of potassium iodide is advisable for reasons given by Bak, Brun and Raaschou. It is necessary to start the bubbling through as soon as the KI has been added in order to prevent atmospheric oxidation. Bilman (12) claims that the bubbling through will cause loss of iodine by sublimation, but own experiments with a solution containing 22 micrograms of iodine gave no loss when the bubbling was moderate and less than 2 % when it was very lively. If kept moderate at first, the bubbling may well be increased towards the end of the titration.

11) Titrate with sodium thiosulfate (reagent 10) until the colour has completely disappeared. White background and yellow lamplight.

Note: N/1000 thiosulfate (reagent j) is added from the above mentioned Krogh-Rehberg burette, with the tip of the burette dipping down into the solution. With an ordinary stopcock burette the solution of thiosulfate must be less concentrated, e. g. N/2000.

Calculations: Consumed ml of N/1000 sodium thiosulfate × 21.16 gives the iodine content of the solution in micrograms, whence it is easy to calculate the iodine content of plasma since the dilution is known.

Testing the method: The present modified method was tested, first of all, on standard solutions prepared by dissolving a dried powder of May and Baker's "Uriodone forte" in heparin plasma. The preparation in question contains also the diethylamin salt besides the diethanolamine salt of 3,5-diiodo-4-pyridone-N-acetic acid. The powder, in 3 analyses according to Baubigny and Chavanne's method, was found to contain 49.2, 50.3 and 49.7 % or an average of 49.7 % iodine, while the manufacturers state the percentage to be 50.86; the difference may be attributable to impurities or to the circumstance that the drying, in spite of the apparently constant weight obtained, was not complete.

The standard solutions contained 23.75, 9.29 and 1.90 mg% iodine. 0.5 ml of each was diluted 21 times ($Zn(OH)_2$ and boiling for 5 minutes), and 4 ml were used except in one series where the volume was only 2.0 ml. The results of the analyses are given in table 1.

Table 1.

Original Iodine Content, Number of Double Analyses, Recovery Percentage and Standard Deviation.

Micrograms of iodine	No. of double analyses	Recovery percentage mean values	Standard deviation
45.2	20	99.7	0.8
17.70	13	98.7	0.6
3.63	10	100.4	0.8
1.81	12	101.4	1.6

The values in all four series were found to be distributed symmetrically about a mean value.

Investigations on urine solutions of the above dried powder showed that the accuracy here was at least equal to that of the determinations in plasma, as evident from the following figures:

Iodine added to urine	Recovery percentage mean values	No. of analyses
47.4 micrograms	99 %	8
1.81 »	100.4 %	8

Summary.

The paper deals chiefly with an investigation of the systematic loss involved in BAK, BRUN and RAASCHOU's modification of WHITE and ROLF's method for the determination of diodrast

(diodone) in blood and urine. An improved analytical method is proposed which suffers neither from blank value nor systematic loss and is accurate within 2.5 % in the iodine interval 4—50 micrograms. It is at least equally as rapid and easy as any previous method, and requires no special apparatus.

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The Fluctuation of pH, Buffer Capacity, and pH-Dependance of Hydrogen Activating Enzyme Systems During Insect Metamorphosis.

By

IVAR AGRELL.

Received 19 February 1948.

Material and Method.

The material under investigation comprises praepupae and pupae of the fly *Calliphora erythrocephala* MEIG. The experimental animals were kept in cultures at + 22° C. The development from pupation to hatching takes 10—11 days at this temperature (cf. AGRELL 1948).

Determination of pH.

The pH-variation in the haemolymph during the metamorphosis has been previously investigated by means of a colorimetric method (AGRELL 1948). It was demonstrated that the pH passes a minimum during the pupal development. Only uncertain values are obtained with the method, however, and electrometrical determinations have now been carried out by means of a glass electrode.

In order to determine pH in the haemolymph MAC INN's microelectrode was used. Every pupa examined was punctured with a thin needle in the dorsal part of the thoracic region, one drop of haemolymph was squeezed out and transferred at once on to the electrode. Each determination lasted 1 minute at the most. No change in pH could be observed when the measuring period was extended up to 5 minutes.

In order to determine the pH in the entire pupa two individuals were crushed in 1 ml. of redistilled water and the measurement was carried out immediately with an ordinary glass electrode. The results are given in fig. 1. Each point represents the average value of 3—5 determinations on different individuals with a maximal mutual deviation of 0.1 pH units.

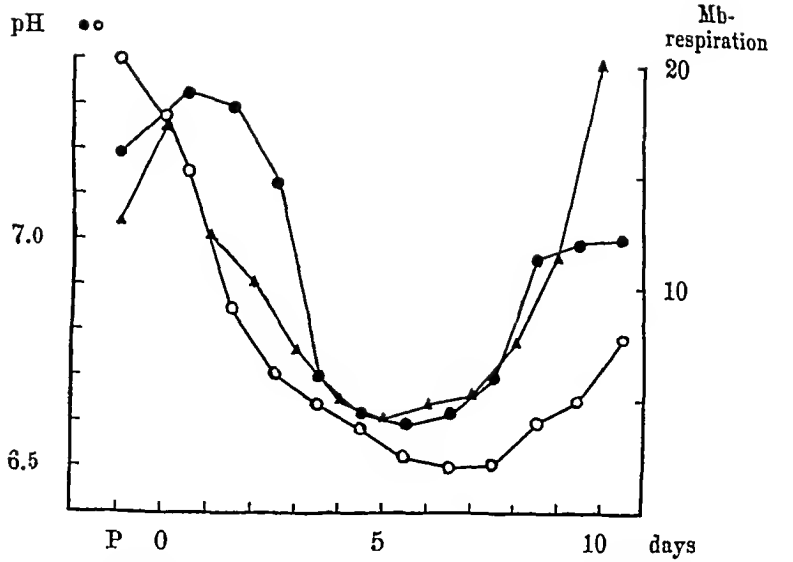

Fig. 1. pH-variation, ● in haemolymph, ○ in pupal matter, and Mb-respiration ▲, during the metamorphosis. Vertical axis, pH-values to the left; spontaneous activity, $\frac{1}{\text{decolorization time}} \times 100$ to the right. Horizontal axis, developmental age. P denotes praepupa one day before pupation.

Determination of the Buffer Capacity.

There was no suitable apparatus for determination of the buffer capacity in blood. These determinations have therefore been carried out only on whole pupae. The following method was used: The cell matter of the pupa is strongly oxidized when exposed to the air due largely to a thyrosinase effect. At the same time the pH decreases rapidly. The usual titration to ascertain the buffer capacity could therefor not be made. The determination of the pH displacement upon the addition of acid and base respectively must take place within a minimum of time. Thus two pupae were crushed in 1 ml. redistilled water and 1 ml. HCl respective KOH of known strength was added, after which the pH alteration was measured directly in the electrode vessel. The measurement was repeated with varied concentration of acid and base. This series of measurements gives a titer curve of the usual type, shown in fig. 2. Here each point, the average value of 3 determinations, represents, however, measurements on different individuals of the same age. Experiments were carried out during the whole developmental period in the same way.

The buffer capacity, β, is defined according to VAN SLYKE 1922 as $\frac{dB}{dpH}$, where dB represents the increase in basic concentration and dpH the increase in pH. An increase in the acid concentration corresponds to —dB and dpH also assumes a negative value. β is thus always a

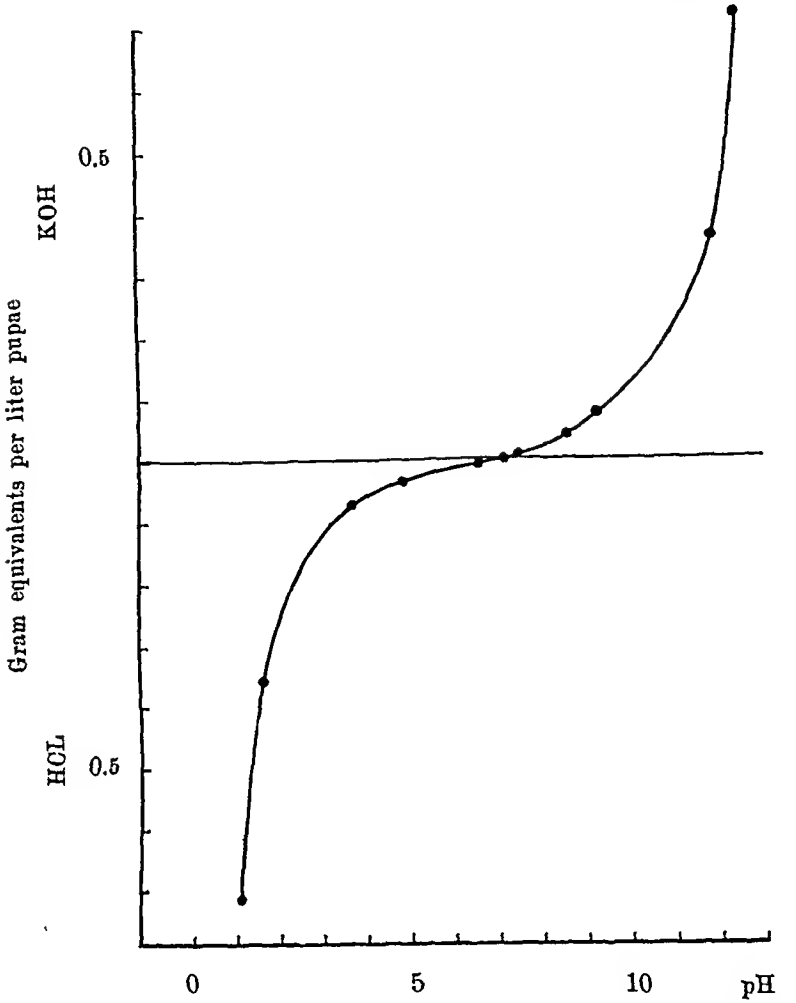

Fig. 2. The change in the pH of the pupal matter, the horizontal axis, with acid or base added, vertical axis. Newformed pupae.

positive number. The buffer capacity 1 indicates a solution which on addition of 1 gram equivalent of acid or base per liter alters the pH 1 unit. In the present case β was calculated graphically partly on the acid and partly on the basic side with 1 liter pupal matter as unit and with the natural, actual pH of the pupa as the initial value. The buffer capacity registered expresses on which side of this pH the β maximum is situated. As the buffer capacity shows β-maximum at pK it is also indicated on which side of the actual pH the active buffer substances' "mean-pK" is situated.

The variation in the buffer capacity during the development is registered in fig. 3. Points, connected with lines, indicate successive measurements within the identically same culture. Remaining points denote isolated measurements.

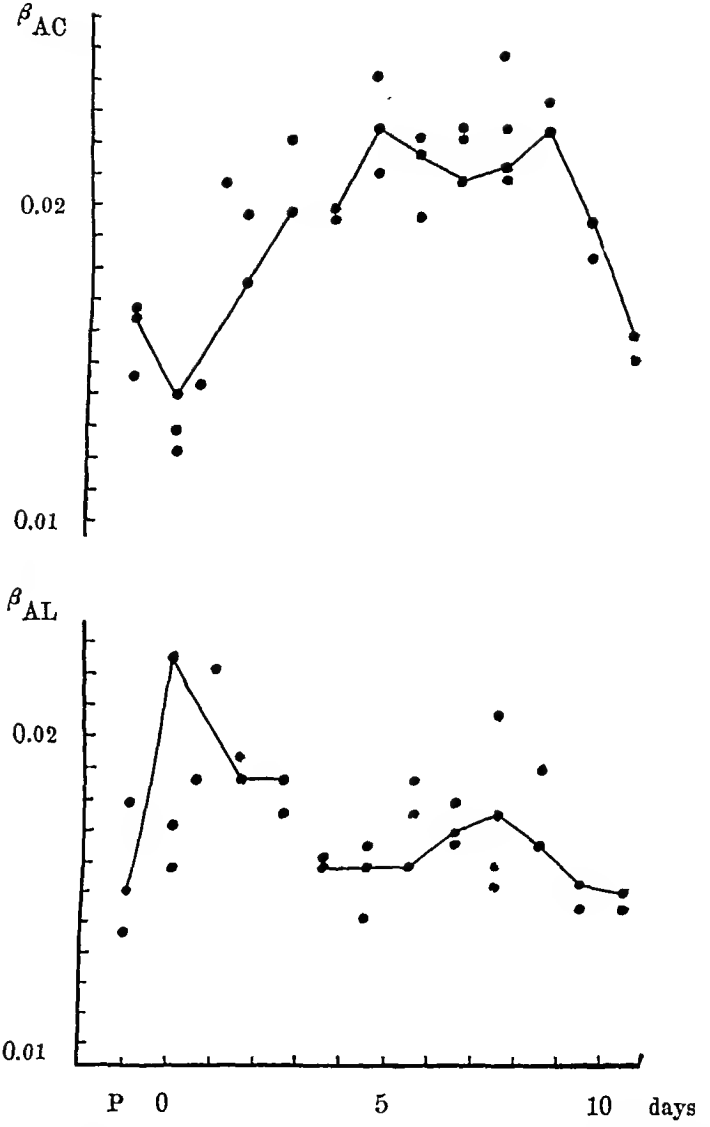

Fig. 3. The change in the buffer capacity, vertical axis, with raising devclopmental age, horizontal axis. $^{\beta}$AC = buffer capacity on the acid side, $^{\beta}$AL = buffer capacity at the alkaline side.

Determination of the pH-dependance of the Mb-respiration.

The rate of anaerobic decoloration of methylene blue, Mb, in THUNBERG-experiments, is used as an expression of the capacity of the hydrogen activating enzyme systems (cf. AGRELL 1948). In every separate experiment one pupa was used crushed in 0.5 ml. of Mb-solution, 1 : 50000, and 1 ml. of buffer. As the buffer a phosphate buffer was used, primary + secondary phosphate in the concentration M/15, and continuous determinations of the Mb-respiration during

the metamorphosis were made at pH 6.0, 6.5, 7.0, 7.5 and 7.8, and also a boric acid-borate buffer of the same concentration with determinations at pH 7.2, 7.5, 8.0, 8.5 and 8.8. Each respiration value was obtained as the average of 8—10 separate tube tests. The resulting curves seemed to reach maxima in the Mb-respiration at pH 8 in phosphate buffer and at pH 7 in borate buffer. Two such series are

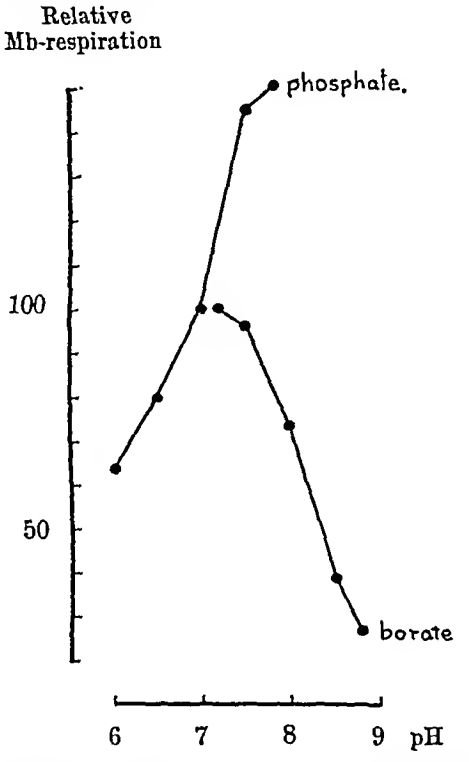

Fig. 4. The pH-activity of the Mb-respiration for 4.5 days old pupae in phosphate buffer, the respiration at pH 7.0 = 100, and in borate buffer, the respiration at pH 7.2 = 100.

exemplified in fig. 4. As has been pointed out before, however, by LAKI (1938) and others, such maxima are abiological, being highly dependant of the redox indicator used and therefore of small interest. The result is also influenced by the buffer substance, as appears from the above. The important thing is, however, to establish possible fluctuations in the pH-activity of the Mb-respiration during the metamorphosis. Such fluctuations are caused by changes in the biological material under investigation, in this case of biochemical changes during the development corresponding to differences in the pH-activity of the natural redox systems at different ages; the pH-dependence in potential of Mb, on the other hand, should be considered as constant. In order to express this relative activity of the enzyme systems in alkalinized and acidified environments respectively, the Mb-respiration at pH 7 was taken as equal to 100 in the experi-

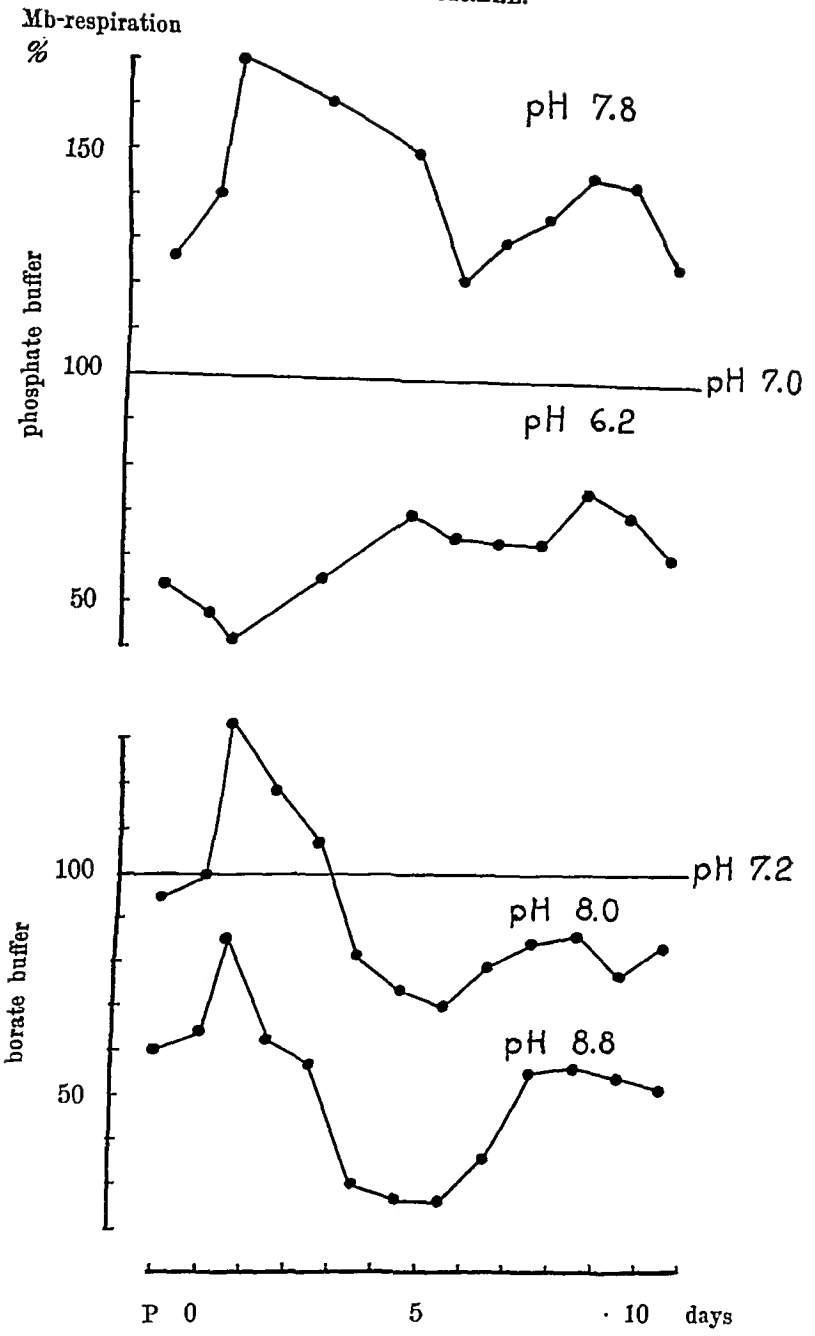

Fig. 5. The change in the pH-activity of the Mb-respiration, vertical axis, with rising developmental age, horizontal axis. For the phosphate buffer the Mb-respiration at pH 7.0 = 100, for the borate buffer the Mb-respiration at pH 7.2 = 100.

ments with the phosphate buffer; the respiration at 6.2 and 7.8 was calculated in percent hereof. Likewise in the results of the experiments with borate buffer the respiration at pH 7.2 was taken as

equal to 100 and the respiration at pH 8.0 and 8.8 was calculated in per cent. The pH-value 7.0—7.2 corresponds approximately to the degreee of acidity at pupation. The results are represented in fig. 5.

Results.

It appears from Fig. 1 that the pH is changed during the metamorphosis of *Calliphora* and passes through a minimum. The pupal matter is always somewhat more acid than the haemolymph. The total pH-variation is remarkable great — between 7.4 and 6.5. The Mb-respiration, fig. 1, and also the gas metabolism (AGRELL 1948) show a similar U-shaped alteration as a function of the developmental age. The rising part of the curve is higher in these cases, however. The U-shape of the respiratory eurve eannot be caused primarily by the natural pH-variation, beeause, among other reasons, the experiments on the Mb-respiration were carried out in buffered solutions with a constant pH, but the Mb-respiration shows a U-eurve inspite of this fact. It is evident from the literature that the relation between the respiration and pH during the inseet metamorphosis need not be parallel. U-formed respiration curves have been established for all holometaboles hitherto examined. A corresponding pH-variation with a minimum during the pupal development has only been reported by FINK (1925) for a Dipteron and a Lepidopteron. LUDWIG (1931 and 1934), for instanee, finds an almost constant pH during the development of *Popilla* (Col.). TAYLOR (1934) has for *Galleria* (Lep.) observed a pH-variation 6.6—5.8 fluetuating inversely to the respiration. DEMJANOWSKI (1932) noted for *Bombyx* (Lep.) a fluetuation within a limited pH-range, 6.9—6.7, resembling most closely the variation in the reducing capacity of the haemolymph during the development (DEMJANOWSKI 1935).

As has been pointed out above, the Mb-respiration, indicator of the capacity of the hydrogen activating enzyme systems, has so far only been examined with a constant pH. The author has therefore studied this respiration at varied pH in order to aseertain whether any alteration in the pH-dependenee of the respiration — the relative activity of the enzyme systems in acidified and alkalinized medium respectively — oecurred during the metamorphosis. The experiments were made with two buffers, one phosphate and one borate buffer. The results from the ex-

periments with the phosphate buffer are of greater interest here as previous respiration experiments were carried out with phosphate buffers, and borate, moreover, has an inhibitory effect on the respiration, which shows a definite fluctuation during the course of development. The results are presented in fig. 5.

It appears from the diagram that the respiration during the metamorphosis shows two relative maxima in alkalinized medium, one at pupation and one towards the end of the developmental period. A minimum occurs at about half the pupal time. In acidified medium there are one relative minimum for the Mb-respiration at the pupation and one, or possibly two, maxima at and after the middle of the pupal time. There is a certain agreement between pH-variation and the pH-dependence of the Mb-respiration during the metamorphosis, figures 1 and 5. There is thus at pupation when the pupal matter is faintly alkaline, a relative maximum for the respiration in alkaline and a relative minimum in acid medium. At about half the pupal time when the pupal matter is definitely acidic there is a relative maximum in acidic environment and a relative minimum in alkaline. In connection with the latter rise in pH there is also an increase in the respiration in alkaline medium. It should be stressed that the natural pH-variation does not cause the altered pH-sensibility of the respiration, the experiments being carried out in buffered solutions.

In might be of interest in this connection to compare with the variation of the buffer capacity in the pupa during the development. This variation is presented in fig. 3. The buffer capacity, as previously mentioned, has been examined partly on the acid and partly on the alkaline side. The average values for the whole developmental period, 0.020 and 0.017, correspond on the whole to the buffer value in human blood and also in insect blood according to, for example, Babers (1941) for a Noctuide larva. The buffer capacity shows a fluctuation during the metamorphosis which is closely parallel to the variation in the pH-activity of the Mb-respiration, fig. 5. Maxima and minima coincide closely. A high buffer capacity on the acid and alkaline sides corresponds respectively to the relative maxima for the respiration in acidified and alkalinized medium and, conversely, a low buffer value corresponds to relative minima for corresponding respiration. The agreement observed is not caused by the fact that the buffer capacity regulates the pH-dependence of the respiration, the respiratory experiments being carried out in artificial buffered

environment. It seems very probable, however, that the metabolism, reflected in the activity of the dehydrogenase systems, determines the buffer capacity.

These facts in connection with the natural pH-variation, Fig. 1, show, that the capacity of the pupa during development, to counteract the effect of the change in acidity on the respiration is dependent both upon the alterations in the buffer capacity and in the pH-activity of the hydrogen-activating enzyme systems. When the pupal matter is alkaline there is a maximum in buffer capacity and relative Mb-respiration in alkaline medium. As the pH of the pupal matter decreases the buffer capacity and Mb-respiration increases in acid medium. In connection with the increase in pH occurring later on, the buffer value and Mb-respiration rise again in alkaline environment. Towards the end of the pupal development the buffer capacity diminishes for both acid and alkaline mediums, which must be due to the fact that the total amount of active buffer substances is reduced. A similar decrease is observed in the relative Mb-respiration.

At this point in the investigation it is difficult to say which substance in the pupa causes the observed buffer effect. According to previous investigations carbonic acid/bicarbonate and phosphate buffers seem to play a relatively insignificant rôle in insects (cf. Babers 1941). Ampholytes, for example proteins and especially amino acids, seem to be of greater importance, the latter are present in high concentraton in the blood of insects (cf. among others Heller 1932 and Ussing 1946).

Experiments on the activity of the dehydrogenase systems have as has been indicated before, been carried out by means of two different types of buffers, one phosphate and one borate buffer. It appeared that the decolorization of Mb occurred more rapidly in the phosphate than in the borate buffer. Two possibilites could be considered. Either an activation through the phosphate which may well be assumed considering the great importance of the phosphorylation during the biological oxidation, or an inhibitory effect through the borate. Experiments with borate buffer and a varying amount of phosphate added at different pH and different stages of development showed, however, that the phosphate, independent of the concentration, was entirely without effect on the Mb-respiration. An addition of borate to the phosphate buffer had an inhibitory effect, however. The inhibitory effect of the borate fluctuates regularly during development and is represented

in fig. 6. The borate inhibition occurs at the same biochemically critical periods during development as found for certain hydrogen activating enzyme systems reported previously (AGRELL 1948). It is, however, at present impossible to establish the exact type of the inhibitory effect of the borate. The pharmacodynamical effect of the boric acid seems strangely enough to be but little known.

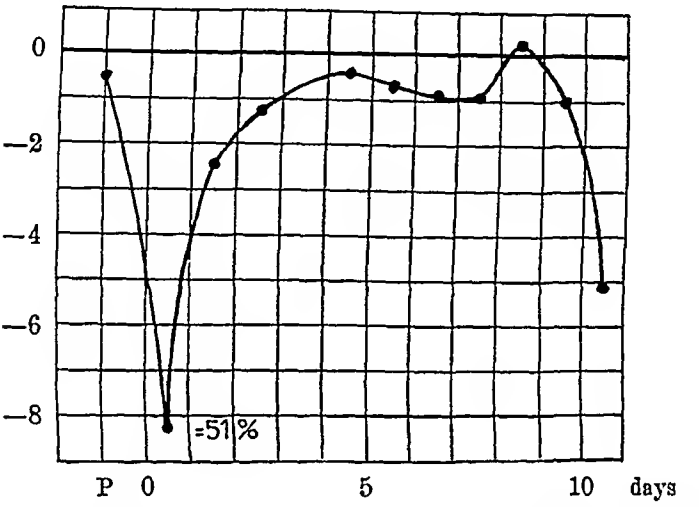

Fig. 6. The inhibitive effect of the borate on the Mb-respiration, vertical axis, with rising developmental age, horizontal axis. The inhibition is expressed as $\left(\frac{1}{A}-\frac{1}{B}\right) \times 100$, where A is the decolorization time for methylene blue in borate buffer and B the decolorization time in phosphate buffer. pH is in both cases 7.2.

Valuable technical work in this investigation has been carried out by Miss ELSA ROSENGREN. The investigation has been facilitated by grants from the Scandinavian Insulin Foundation and from the Swedish Government Research Board for Natural Science.

Summary.

1. pH, buffer capacity and dehydrogenase activity have been examined in pupae of *Calliphora erythrocephala* MEIG during the entire metamorphosis.

2. The pH-variation as a function of the developmental age represents a U-curve of about the same type as the respiratory curve. A primary effect of the natural acidity on the course of the respiratory curve is out of the question.

3. The buffer capacity in pupal matter is of about the same magnitude as in human blood. The buffer value on the acid and alkaline side respectively and the methylene blue respiration in acidified and alkalized medium respectively fluctuates during the development parallel. The pH-activity of Mb-respiration is indeed independent of the natural buffer capacity. There is reason to assume, however, that the variation in buffer capacity is determined by changes in the activity of the hydrogen activating enzyme systems.

4. The capacity of the pupa to counteract the effect of the change in acidity on the respiration during development depends on both changes in the buffer capacity and in the pH-activity of the hydrogen activating enzyme systems.

5. A borate buffer has in comparison with a phosphate buffer an inhibitory effect on the Mb-respiration. This inhibition fluctuates during development in a regular manner, accentuating the occurrence of biochemically critical periods during the development, which have been demonstrated previously.

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From the Institute for Research in Organic Chemistry,
University of Stockholm.

Note on the Inorganic Phosphate of Blood Plasma.

By

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Received 23 February 1948.

In tracer work with radiophosphorus we often add labelled sodium phosphate to blood plasma or inject it into the circulation and follow by means of radioactive measurements the path of the tagged phosphate into the red corpuscles, their passage through the capillary wall or other phase boundaries. For this type of work it is of great importance to know whether the inorganic phosphate, added or injected, shows the same behaviour as the endogenous inorganic phosphate present in the plasma. Should that not be the case, the calculation of the amount of phosphate penetrating from the plasma into the red corpuscles, for example, from the distribution date of the ^{32}P added and the inorganic phosphate of the plasma would clearly lead to wrong results. To investigate if and to what extent added and endogenous inorganic phosphate show a different behaviour we added to heparinized human or cat plasma a tracer dose of sodium phosphate of negligible weight and, after rotating the plasma at 37° for 1 hour in a thermostat, we electrodialyzed the plasma. At intervals varying between 1 and 18 hours, we took samples and determined by radioactive measurements and colorimetric determinations, respectively, at what rate the added labelled phosphate and the endogenous inorganic P, respectively, is removed from the plasma.

Experimental.

The dialysator applied was of the Hahn-Tiselius (1943) type. As membrane a thin sheet of cellophane was used. To 20 ml heparinized plasma an equal volume of physiological sodium chloride solution containing about 10 γ labelled phosphate of $^1/_2$ microcurie activity was added. To keep the salt content of the plasma at constant level a diluted sodium chloride solution was added at intervals. Through the electrode cells a borate buffer solution of pH 7.6 circulated. The potential applied to the ice-cooled dialysator was 20 Volts; the current intensity amounted to 30 milliamp. The inorganic phosphate was extracted from the plasma with 10 % trichloroacetic acid. We carried out extractions both at 0° and at 20°. Imperfect extraction would not influence our results, as aliquots of the same extract were applied to the radioactive measurements and to the colorimetric determinations.

In some cases, the amount of the total acid-soluble P present and its activity were determined as well. In these experiments, the acid-soluble extract was ashed and an aliquot used to colorimetric determination of the total (inorganic + organic) acid-soluble P present, another aliquot to radioactive measurements.

Results.

Numerous experiments were carried out leading to the result that a difference is present between the behaviour of the inorganic phosphate added and the endogenous inorganic phosphate, but the difference is not pronounced. As seen in Fig. 1 and other similar figures obtained by us, the radioactivity of the plasma sample declines at a somewhat more rapid rate than its inorganic P content. After the lapse of 18 hours 10 % of the inorganic P is still present, but only 4 % of the activity added. Thus, about 6 % of the endogenous plasma inorganic P do not dialyze and, consequently, do not get into exchange equilibrium with the labelled phosphate of the plasma. In some experiments, up to 10 % of the endogenous inorganic P were found not to dialyze. The non-dialyzing inorganic phosphate is presumably combined with proteins. That a minor part of the inorganic P of the plasma is bound to proteins is also made very probable by previous work in which other methods were used and which are discussed below.

As seen in Table 1, the total acid-soluble P content was found to be appreciably higher than the inorganic P content, about 1/5 of the acid-soluble P being present in organic binding. The specific activity of the total acid-soluble P was found to be lower than the

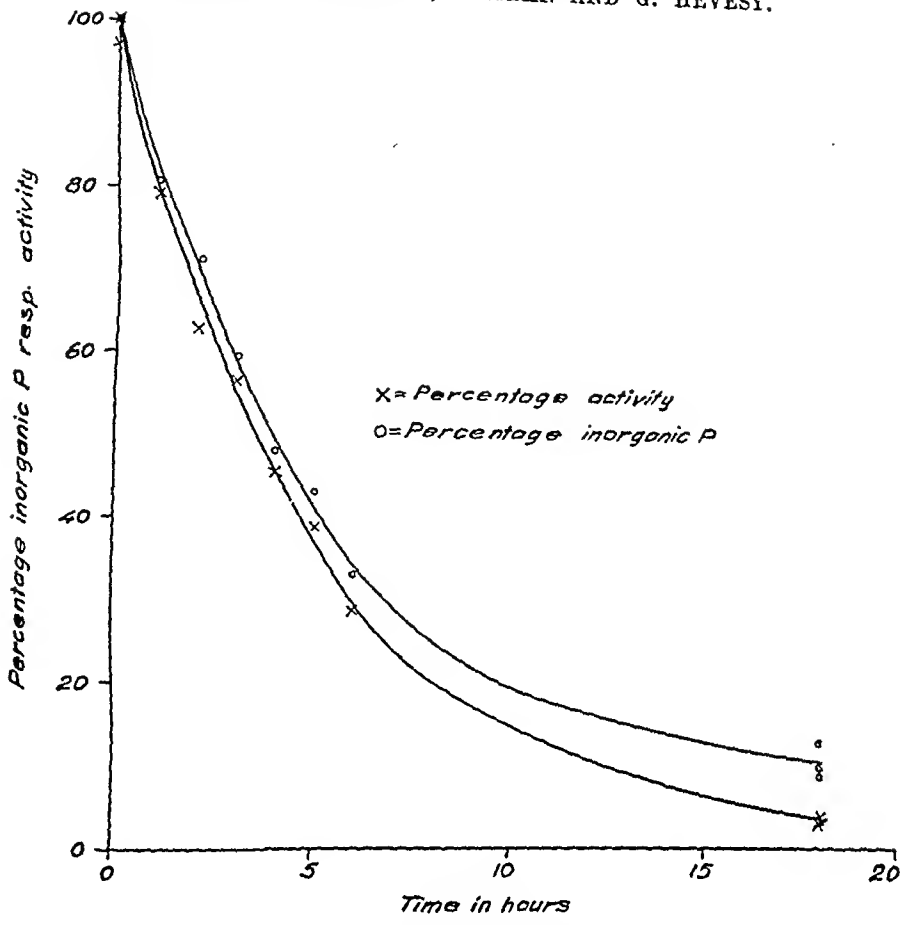

Fig. 1. Rate of disappearance of the endogenous inorganic P content of human plasma and of the radiophosphorus added.

corresponding value of the inorganic P, indicating that the labelled phosphate does not interchange or interchange to a minor extent only with the organic P present in the plasma.

Previous Work on the Presence of Phosphate-Protein Compounds in the Plasma.

MACHEBOEUF and SØRENSEN (1925/27) in their studies on the composition of egg-albumin were led to the result that between the large complexes which are constituting egg-albumin molecules few phosphorus containing complexes are present; these are bound so firmly to the other groups of the molecule that they have to be considered a constituent of the latter. The amount of protein-bound phosphorus was found in albumin, resp. globulin to amount to 7,5, resp. 2 mg per g total nitrogen. This phosphorus

Table 1.

Inorganic P Content and Activity of Plasma Samples Secured from the Dialysator at Various Times.

Time in hours	Inorganic P content in relative units (100 relative units = 3.7 mg%)	Activity in relative units (100 relative units = 200 counts per min.)
0	100	100
2	69.3	63.2
3	58.5	56.7
4	47.1	45.6
5	42.6	39.2
6	32.7	29.3
18 [1]	9.8	3.2
18 [1]	9.1	3.9
18 [2]	12.7	4.4
	Total acid-soluble P	Specific activity of total acid-soluble P
		Specific activity of inorganic P
0	127	0.80

could not be separated from albumin, resp. globulin, by electrodialysis. SØRENSEN (1925/27) investigated on similar lines the phosphorus content of serum-albumin and serum-euglobulin. Serum albumin and euglobulin were found to contain 2—40, resp. 0.15—0.3 mg P per g total nitrogen. In contrast to the phosphorus of egg proteins only a small percentage of the phosphorus of the serum proteins could be precipitated by alcohol. This and other observations induced SØRENSEN to regard the serum protein P as an accessory constituent of the serum proteins only. No conclusions can be drawn from these investigations if this accessory phosphorus is getting into exchange equilibrium with the comparatively large amounts of inorganic phosphorus simultaneously present in the plasma or not.

MASKET and associates (1942) analysed fractionated centrifuged horse serum and found that the inorganic phosphorus concentration increases progressively with the protein concentration. Differences of 0.14 and 0.15 mM of P per kilo water were obtained between the top and bottom fraction, the top fraction containing 11 % less, the bottom fraction 12 % more inorganic P than the unfractionated plasma which contained 1.3 mM per kilo of H_2O.

[1] Extracted at 20°.
[2] Extracted at 0°.

These authors conclude from their results that phosphate-protein compounds normally occur in horse serum.

That protein bound inorganic phosphate is present in small amount only in the serum of the dog is also borne out by the work of SMITH et al. (1943) who determined the percentage of ultrafilterable inorganic serum phosphate in 13 cases. The average percentage of ultrafilterable inorganic P found in their experiments works out to be 96 %.

Not only a combination with proteins, but also the formation of colloidal calcium phosphate will prevent ultrafiltration of inorganic P or its removal into the electrodialysate. GROLLMAN (1927) found in an early work that, while the inorganic P of normal pig serum is entirely ultrafilterable, a successive increase in the calcium content of the plasma from 9.4 to 32.2 mg % makes the inorganic phosphate less and less ultrafilterable and, finally, only 5 % are found in the ultrafiltrate. Ample evidence was brought by different authors that only excessive quantities of calcium phosphate salts lead to the formation of detectable amounts of a colloid complex (cf. SCHMIDT and GREENBERG, 1935; MCLEAN and HINDRICHS, 1938).

GOVAERTS (1943, 1947) compared the specific activities of the inorganic P of plasma and urine shortly after intravenous injection of labelled phosphate into the dog. In the first 1 ½ hours, the specific activity of the urine P was found to be greater than the corresponding value of the plasma P; after the lapse of that time no difference was found. GOVAERTS interprets these results as indicating that the greater part of the acid-soluble P of the plasma does not get into exchange equilibrium with the injected inorganic phosphorus.

Our results do not contradict those of GOVAERTS. Phosphate identified after treatment of the plasma with trichloracetic acid as inorganic phosphate but actually present in the plasma in a labile low molecular organic binding may show a similar electrodialytic behaviour as does inorganic phosphate. Proofs of the presence of a labile phosphorus compound in the plasma is yet outstanding.

Summary.

To determine whether inorganic labelled phosphate added to plasma gets into exchange equilibrium with inorganic phosphate present previously, labelled phosphate of negligible weight was

added to human plasma. The plasma was electrodialyzed, samples were taken from the dialysator at intervals, their inorganic phosphorus content and its radioactivity determined.

No pronounced difference was found in the rate of disappearance of the inorganic P content and of the radioactivity of the sample. As, however, after the lapse of 18 hours, only 4 % of the original activity, but 10 % of the original inorganic P content were present, we have to conclude that a small percentage of the plasma inorganic phosphate, possibly combined with proteins, does not interchange with the labelled phosphate added.

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A Note on Optical Specificity in Methylation Processes.

By

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Received 1 March 1948.

The effect of optical isomerism in methylation processes seems to have been only little studied. As far as the present writer is aware the only investigation pertinent to this problem is that of HANDLER and BERNHEIM (1943), who compared the relative efficiencies of the optical isomers of methionine when acting as methyl donators in the methylation of guanidine acetic acid to creatine in the presence of liver slices in vitro. They found d(+)-methionine to be about 50 per cent as active as the natural isomer. It has therefore to the present writer seemed not uninteresting to carry out some further work on this problem. The first results of these studies will be reported in the present note, in which it is attempted to investigate the rôle of optical isomerism in the enzymatic conversion of arginine and histidine to creatine. A review of much of the literature on this process has been given, for instance by GUGGENHEIM (1940) and by MENNE (1942).

Experimental Results and Their Discussion.

Samples of l(—)-, dl- and d(+)-histidine were obtained from F. Hoffmann-La Roche & Co. A fine sample of dl-arginine was kindly presented to me by Professor MAX DUNN. A small sample of the latter compound, in sufficient quantity for use in some preliminary experiments, was also synthesized from ornithine by the writer according to the well known method of SØRENSEN (see SCHMIDT (1938)).

The biological material was frog muscles. The animals were killed by decapitation, and the muscles from the hind legs removed im-

mediately afterwards. They were finely divided on a watch glass by means of a pair of bent scissors. The resulting pulp could be conveniently handled and weighed with sufficient accuracy. This crude enzyme preparation is sufficient for the problem under consideration.

A typical experiment with histidine was carried out as follows. A glass vessel A contained

0.4 g muscle tissue
6 ml phosphate buffer (pH 7.0)

Another vessel B contained

0.4 g muscle tissue
4 ml phosphate buffer (pH 7.0)
2 ml of phosphate buffer solution of l(—)-histidine monohydrochloride (adjusted to pH 7.0 by the addition of alkali).

Vessels C and D were both prepared in identically the same way as vessel B, but with l(—)-histidine replaced by the dl- resp. d(+)-isomers.

The vessels were left standing at room temperature for 3 hours, under occasional shaking and stirring. After this period 4 ml 10 per cent trichloroacetic acid and 2 ml n/1 HCl were added. After standing for 1 hour the mixtures were spun down in a centrifuge. 5 ml of the supernatant liquid were then autoclaved for 20 minutes at 130° C. After cooling to room temperature a small quantity of methyl red indicator was added, followed by an amount of 20 per cent sodium hydroxide solution sufficient to ensure exact neutralization. Water was now added to bring the volume of the liquid to 11 ml. We then added 10 ml of a 6 per cent aqueous solution of sodium-3,5-dinitro benzoate, 10 ml 20 per cent sodium acetate and 1 ml 10 per cent sodium hydroxide solution. After standing for 5 minutes water was filled up to the mark in a 50 ml flask. The photometric measurements were carried out in the ordinary way in a Pulfrich photometer. This is essentially the BENEDICT-BEHRE-LANGLEY-EVANS-LEHNARTZ method for the determination of total creatinine. It is unaffected by the presence of histidine and arginine. The method has been previously applied by the present writer (STEENSHOLT 1946).

Duplicate experiments and duplicate analyses were of course carried out both here and in the sequel.

Table 1 gives the results obtained in 5 experiments with muscles from different animals. We give only the percentage increase in total creatinine in the vessels B, C and D compared to vessel A.

Similar experiments were also carried out at different hydrogen ion concentrations. The results of two experiments are recorded in Table 2. In each experiment the muscles from 3 different frogs were used, the muscles being lumped together and minced as described above. All in all five experiments of this type were carried out, but the numerical details for two of them will probably suffice to indicate the nature of the results.

It should be remarked that the results obtained for d(+)-histidine (last columns in Tables 1 and 2) are rather uncertain. Our method is not accurate enough to permit us to pronounce with certainty upon such small values of the increase in total creatinine.

Table 1.

Experiment No.	Relative increase in total creatinine in per cent		
	Vessel B	Vessel C	Vessel D
1	14	6.5	1.1
2	12.5	7.1	0.4
3	16	7.5	0.5
4	11.5	5.4	0.0
5	14.2	6.2	0.0

Table 2.

pH	Relative increase in total creatinine in per cent.					
	Vessel B		Vessel C		Vessel D	
6.0	4.0	3.7	2.5	2.0	0.1	0.2
6.4	7.0	6.2	3.8	3.1	0.2	0.0
6.8	10.0	9.7	4.8	5.2	0.0	0.0
7.0	12.0	11.5	6.5	6.0	0.2	0.1
7.2	9.8	11.0	5.0	4.8	0.4	0.1
7.7	7.0	7.1	4.1	3.8	0.2	0.0
8.4	4.0	4.3	2.5	2.3	0.0	0.0

A similar series of experiments was carried out with arginine replacing histidine. As already mentioned only the naturally occurring optical isomer (commonly called d-arginine) and dl-arginine were available. Vessel A was prepared as above, and in vessels B and C histidine was replaced by d-arginine and by dl-arginine respectively. In Table 3 we give some typical results obtained at pH 7.0 and with 40 mg of d- resp. dl-arginine.

These experiments were also repeated at various hydrogen ion concentrations. The pH dependence of the effect under consideration was found to be similar to that observed above in the work with histidine. A maximum increase in the total creatinine was found for pH around 7.0, and the relative values obtained with d-arginine and dl-arginine were very nearly those shown in Table 3. It is therefore probably superfluous to give further numerical details of these experiments.

Table 3.

Experiment No.	Relative increase in total creatinine in per cent	
	Vessel B	Vessel C
1	8.6	4.3
2	6.8	3.6
3	7.9	3.8
4	10.0	4.7
5	11.1	5.7

In some further experiments, both with histidine and arginine, we varied the relative amounts of tissue and substrates. However, we do not go into the numerical details of these experiments, since their qualitative character was in complete agreement with the work already reported. It may perhaps be mentioned that among the animals used in the present work we found only one, the muscle tissue of which failed to effect the transformation of histidine and arginine to creatine. The reason for this failure could not be ascertained.

It is clear from the experimental results for histidine reported above, that only the »natural» optical isomer is transformed into creatine by incubation with frog muscle tissue. The other optical isomer appears to be practically unaffected, and neither does it interfere with the transformation of the natural isomer to creatine. At the present time it seems to be very difficult to say with which intermediary reactions in the transformation histidine—creatine this effect of optical isomerism is associated. We are at present very ignorant of the chemical mechanism by which the conversion in question is effected. The similarity in chemical structure between histidine and creatine is of course well known, both being a kind of imidazole derivatives. It is possible, by way of hypothesis, to write down chains of intermediary reactions leading from histidine to creatine, but it is impossible on the basis of our present experimental material to decide for or against these various hypotheses. The enzyme chemistry of the transformation is likewise practically unknown.

From the experiments reported above we can further conclude that only the »natural» optical isomer of arginine is transformed to creatine by frog muscle tissue in vitro. The other isomer seems to be inactive, or very nearly so, and does not interfere with the conversion of its antipode to creatine. If we assume that the conversion of arginine to creatine is effected by transforming arginine

to guanidine acetic acid via γ-guanidine butyric acid, with the subsequent methylation of guanidine acetic acid to creatine by means of suitable methyl donators, then it appears likely that the optical isomer effect reported in the present note is associated with the first step in this chain of reactions, *i. e.* the step arginine → γ-guanidine butyric acid. However, at the present time definite experimental proof for the correctness of this explanation may be lacking (see for instance GUGGENHEIM, l. c.).

It is hoped to discuss other aspects of the histidine—creatine transformation in a future note.

I am glad to express my grateful thanks to Professor RICHARD EGE for his generous hospitality.

Summary.

The present note reports the results of a study of the effect of optical isomerism in the transformation of histidine and arginine to creatine. It is found that only the naturally occurring optical isomers of both amino acids are converted into creatine by frog muscles in vitro.

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Depressor Reflexes from the Heart.

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The afferent fibres conducting from the heart have recently been the object of much interest. Thus attempts have been made to localize the receptors in the heart in order to get an anatomical basis for the further investigation of their physiological action. There are principally two functions of importance here. First comes their part in eliciting cardiovascular reflexes and secondly the problem of the conduction of pain from the heart. The present investigation deals principally with a study of the impulse traffic in the nerve branches running from the vagal stem to the heart, which experiments were carried out in order to throw some further light upon the localizations, the mode of action of the endings of these afferent fibres and particularly to form an opinion of their reflex response.

The original concept of a cardiovascular reflex mechanism elicited from the heart can first be found in the classical paper by Cyon and Ludwig in 1866. They write: "Die einzelnen Stücke des Circulationsapparates passen sich gegenseitig an . . . denn durch ihn vermag der wesentliche Motor des Blutumlaufes die Wiederstände zu regeln, die er selbst überwinden soll. In dieser Beziehung darf man, ohne voreilig zu sein, aussprechen, dass das Herz, wenn es aus Mangel an Propulsionskräften oder aus überschüssigen Zufluss überfüllt oder in Folge davon gereizt wird, nicht bloss seine Schlagzahl ändert sondern auch den seiner

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Entleerung entgegentretenden Widerstand herabsetzen wird." These authors believed that afferent fibres of the depressor nerve which they just had discovered run down to the heart itself. It was first shown by KÖSTER and TSCHERMAK (1902) that the afferent fibres of the depressor nerve ends in the walls of the aorta, which more recently was substantially confirmed by NONIDEZ (1935) and MURATORI (1936). For that reason the heart itself was for a period generally not considered to be the seat of origin for any depressor reflexes and the discovery of the depressor reflex elicited from the carotid sinus still more deviated the attention to more peripheral seats of origin of depressor reflexes on the arterial side.

Already in 1867 VON BEZOLD and HIRT described a powerful depressor action exerted by veratrine, which action they maintained was reflexly elicited from the heart by afferent fibres running in the vagus stem. The conception of afferent depressory fibres localized in the heart itself was later on, however, mostly abandoned by the above related discovery of the aortic origin of the depressor nerve impulses.

In 1915 BAINBRIDGE describes a reflex from the right side of the heart, which however accelerates the heart when the venous pressure rises. By infusing saline in the veins he found an acceleration of the heart which disappears after section of the right vagus nerve on the neck. This finding has been supported by BOUCKAERT and PANNIER (1942). TITSO (1937 and 1939), however, made very serious objections against the reflex nature of the phenomenon described. He was able to produce the same effect on isolated mammalian heart. He also showed that the fact that the cutting of the right vagus abolishes this acceleration of the heart can be interpreted in quite another way as the heart-rate increases so much after section of the vagus that the effect cannot be produced any longer. The effect can however be produced even after section of the vagi, provided the peripheral end is stimulated strongly in order to reduce the heart-rate to a normal value. He concludes therefore that the acceleration produced by raised venous pressure or aurical distension is due to a local action directly on the sino-auricular node.

The different opinions as regards the existence of the BAINBRIDGE reflex are in our opinion very understandable. The various results may depend upon the use of different anesthetics. It must also be dependent on the actual arterial blood pressure, the conditions of the depressory reflex mechanisms etc. WIGGERS (1945)

for instance does not accept the idea of an accelerating reflex from the heart. He has never found any acceleration following upon injections in the veins.

Since 1937 JARISCH and coworkers have presented very substantial evidence for the view that depressor reflexes can be elicited from the heart itself. This opinion was the outcome of pharmacological findings, which could not be interpreted in any other way. This author found that veratrine and mistle-toe extracts produce a very marked fall in blood pressure and a deceleration of the heart. This phenomenon is accompanied by typical peripheral vasodilation — reciprocal inhibition of the sympathetic outflow. He could definitely prove that the concerned afferent impulses running up in the vagus nerve originate from the heart itself and not from the aortic region.

The postulated impulses were also directly demonstrated by JARISCH, AMANN and SCHAEFER 1942, who recorded increased afferent activity in the right vagus under the action of veratrine. A more detailed study of the afferent outflow in the vagal nerve branches running to the right heart was subsequently made by AMANN and SCHAEFER (1943). Heart synchronous impulses had previously been recorded from strands of the vagus nerve on the neck by ADRIAN (1933) and by PARTRIDGE (1939). Recently WHITTERIDGE (1945, 1947) has isolated afferent fibres in the vagus in the neck responding to the venous pulse as well as to ventricular ac ivity.

Technique and Procedure.

The operation: All experiments were performed on cats anaesthetized by intramuscular injection of 0.05 g chloralose and 0.25 urethane in 7 cc Ringer solution per kg body-weight. In order to get access to the nerve the chest was widely opened and on the side of the experiment the ribs were cut as close to the vertebral column as possible. Mostly the whole of the lung on the side experimented upon was ligated by the lung root and removed. The nerve branches running out from the vagus stem towards the auricle could best be dissected out on the right side after ligating and cutting the azygos vein. Mostly there were two or three strands of nerves running to the auricle on both sides but not infrequently there was only one branch. In order to obtain the highest possible signal-to-noise ratio the common nerve sheath was drawn off starting from the vagus stem. This procedure was found to be very important in order to study the fibre activity in more detail as even the biggest fibres in these nerve branches are

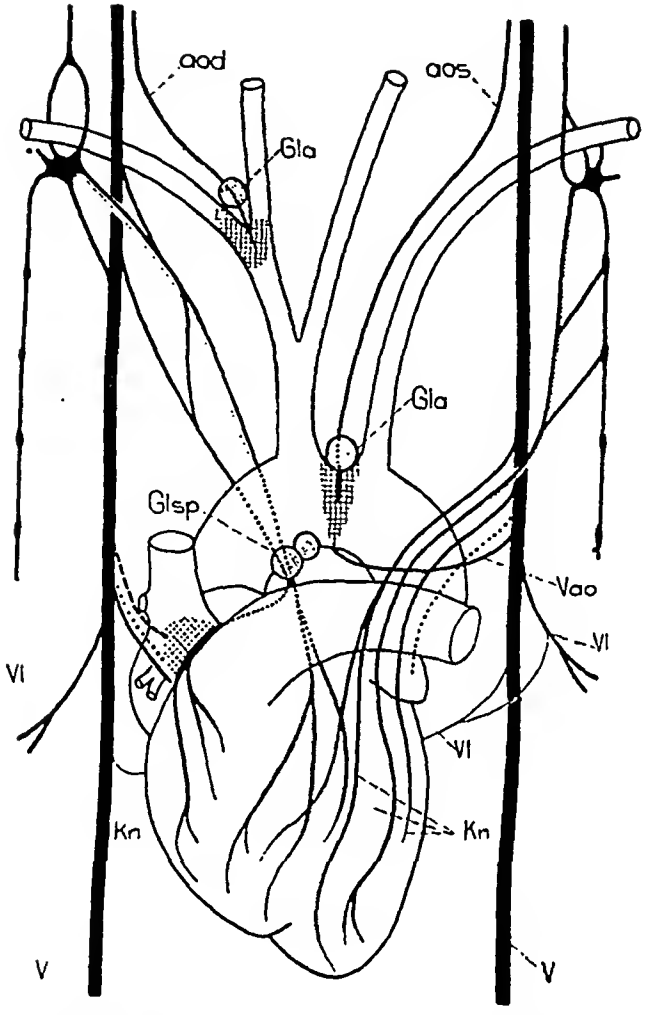

Fig. 1. General arrangement of nerves to the heart. +++ Efferent inhibitory vagal fibres; — — — afferent fibres subserving the Bainbridge reflex; ..... pain fibres. ▒ Receptive field of the aortic depressor nerves. ▨ Receptive field of the Bainbridge reflex. Vn vagus; Kn ventricular fibres. Vao vagal branch to the aorta; Vl vagal branches to the lungs. Vt lower vagal branches to the heart. Aod, Aos right resp. left aortic depressor nerve with Gla, aortic bodies. After JARISCH (1940).

fairly thin fibres presenting relatively small spike potentials. A great obstacle to be overcome is due to the movements of the heart and the lungs which displace the electrodes which must be applied close to the heart on these short nerve strands. In order to obtain a more quiet preparation we usually excised the stellate ganglions. Further the animal must be kept well insulated from the earth in order to avoid that the electrocardiogram will break in. One must keep in mind that the ECG is of an order of c:a 1,000 times higher voltage then are the action potentials led off from the nerve.

Recording systems: The action potentials from the nerve were recorded by a resistance-capacity coupled amplifier previously described ZOTTERMAN (1936) which has an internal noise level of only 1μ volt. The ECG was recorded with the electrodes directly on the heart by the aid of a balanced input amplifier connected to the second ray of the cathode oscillograph tube. The input of this ECG-amplifier had to be very carefully insulated from the earth in order to avoid that the ECG broke through into the nerve potential amplifier. An unexpected connection to earth was produced for instance by the action of pilocarpine which stimulated the salivation and produced

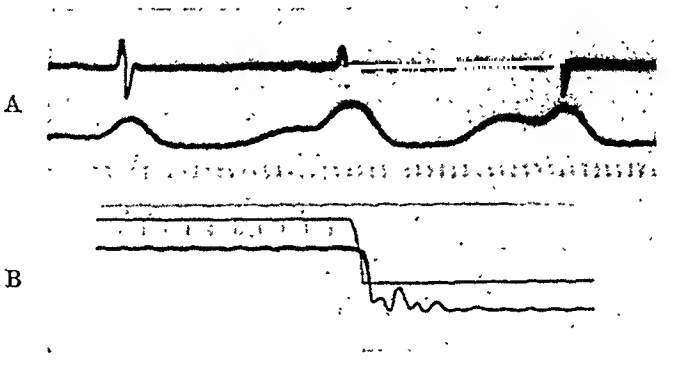

Fig. 2. *A.* Simultaneous record of ECG and pressure changes of right auricle by means of condensor manometer. Time 50 cps. *B.* Calibration of pressure-recorder; upper curve shows the actual change; lower curve: the change recorded by means of the condensor manometer supplied by a 250 mm long tube of an internal diameter of 3 mm as used in the actual experiments. The pressure induced by a syringe was 50 mm H_2O and the change was recorded by means of a variable resistance moved by a lever which was coupled to the syringe piston. As is seen from the record there is a delay of 1/100 of a second but otherwise the pressure recording follows very well the actual change.

a conducting path of saliva to the shielded box surrounding the animal.

The intraauricular pressure was recorded by means of an electrical method designed by T. SÖDERBERG, Copenhagen. It consists of a condensor placed in a high frequency circuit. The phosphobronze membrane — constituting the movable plate of the condensor — is acted upon by the blood pressure and the pressure changes are thus allowed to modulate the high frequency, which is then rectified and amplified by a direct-coupled amplifier. The pressure in the auricle was transmitted by a c:a 20 cm long tube of an internal diameter of 3 mm which was inserted via the subclavian vein down to the auricle. A few drops of heparine in the tube were sufficient to avoid coagulation during the whole experiment. The full sensitivity of the pressure recording system was very high and we generally used only a tenth of the full sensitivity, when a pressure of 30 mm H_2O gave a deflection of 10 mm on the film. The deflections on the screen were calibrated against an H_2O-manometer and the sensitivity desired

could be adjusted by a decade potentiometer regulating the amplification. How well the recording system follows the actual event can be seen in Fig. 2, where a small syringe and a variable resistance were moved by the same lever system. The upper tracing gives the true rate of the event and the lower that of the pressure change transmitted by the tube to the condensor manometer. As will be seen in Fig. 2. the pressure change follows very closely the actual change. There is a delay of only 1/100 sec.

Results.

The behaviour of the auricular receptors. The records of the action potentials led off from the nerve strands on the right side generally show two distinct volleys of impulses at each heart cycle. From simultaneous recordings of the ECG (fig. 4 A) it can be seen that a shorter and sharper volley follows directly upon the P-elevation of the ECG and ends abruptly with the appearance of the QRS-complex. This is a very constant phenomenon. The second volley generally varies a good deal in regard to its intensity as well as to its duration. Usually the second volley starts somewhat before the T-elevation and ends a bit before the P-elevation. Thus there is generally as in Fig. 4 A a very distinct silent period during the phase of isometric contraction of the ventricles. Sometimes there is a second silent period just before the P-elevation. From the records it can thus be seen that the more brief volley coincides with the auricular systole. Then follows a pause during the isometric ventricular contraction. At the end of the expulsory phase the second volley starts and it reaches its maximum intensity just at the end of ventricular systole. Thereafter it may fade away before the next volley sets in or just continue until the next auricular volley starts.

The picture obtained from the corresponding branches running to the left auricle is principally of the same character (Fig. 3 A). There are two volleys of impulses of which the auricular volley is of shorter duration and is generally more marked. The second volley varies much more in intensity and duration by various factors.

Variations in the auricular pressure besides those occurring spontaneously due to the heart activity can be introduced by various means. With the chest open, as in our experiments, the easiest means of introducing gross changes in the auricular pressure

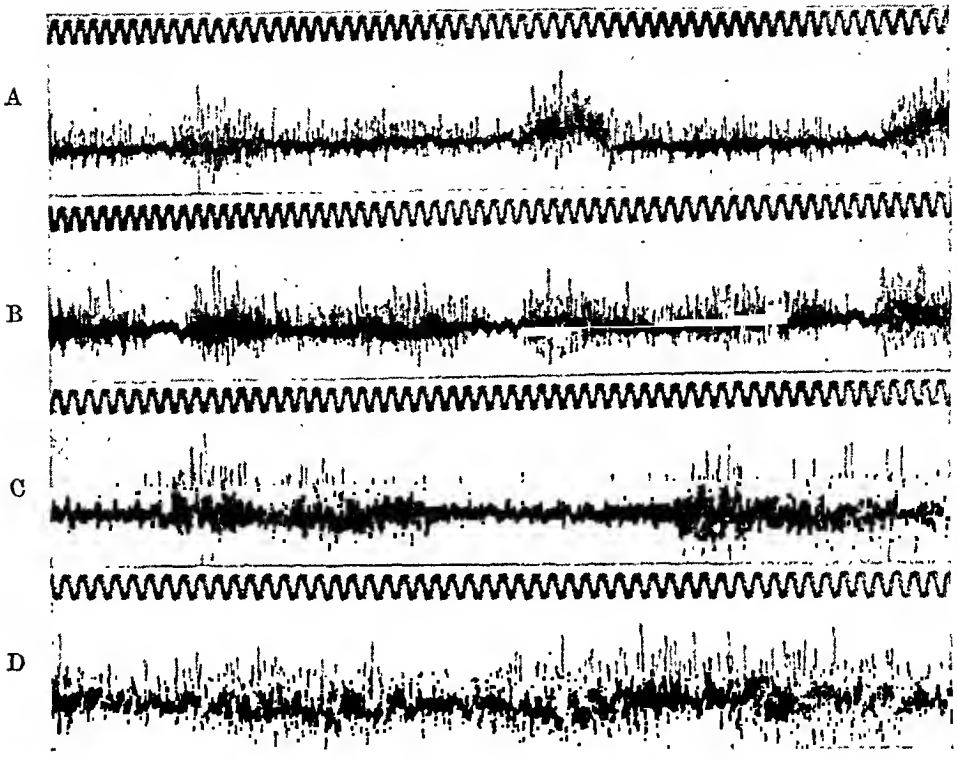

Fig. 3. Action potentials from the vagal branch running to the *left* auricle. *A*. Control; *B*. After raising the venous pressure by injection of 10 cc Ringer. *C*. After 50 γ veratrine intravenously. *D*. The effect of 20 γ adrenaline on the endings sensitized by veratrine. Time 50 cps.

were by 1) clamping the caval veins, 2) clamping the pulmonary artery and 3) clamping the aorta.

As will be seen in Fig. 4 B the clamping of both caval veins which causes the heart to empty is followed by a disappearance of the second volley but does not change the auricular volley. This is a finding, which was quite surprising. It shows, however, that this volley although coinciding with a rise in auricular pressure cannot be elicited by that event but must have some other origin. On release of the caval veins there was at first a very marked increase in the ventricular volley but the auricular volley was hardly influenced (Fig. 4 C).

Clamping the pulmonary artery produces on the right side an augmented activity in the nerve (Fig. 4 D and 7 B) while it exerts the opposite effect on the left side as was expected. When the aorta was clamped there was on both sides a very massive, nearly continuous discharge of high frequency. On release the second volley at first nearly disappears to return gradually to its normal

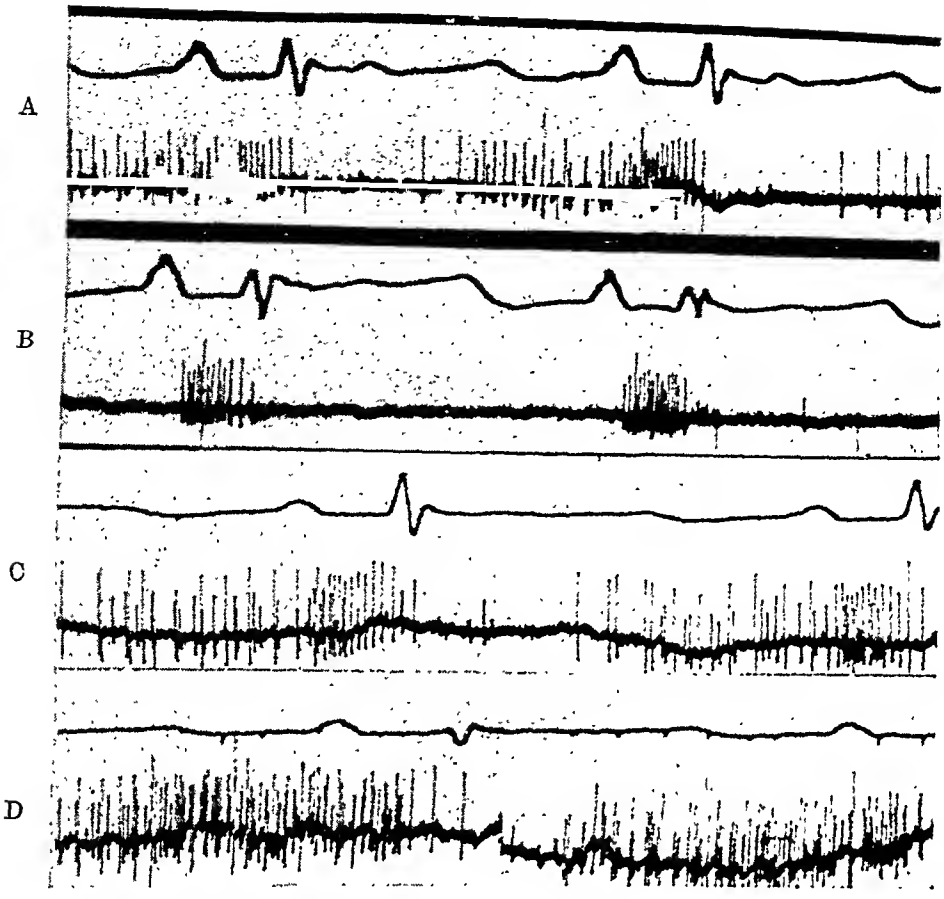

Fig. 4. Simultaneous records of ECG and action potentials from the upper vagal branch to the right auricle. *A*. Control; *B*. After clamping both caval veins; *C*. Caval veins opened again. *D*. After clamping the pulmonary artery.

intensity. This second volley was thus found to be very dependent upon the filling of heart. It varied also with the respiration. Thus with the chest open it increased on the right side with each inflation of the lung.

A rapid injection of body-warm Ringer solution in the femoral vein always produced a very augmented response (see Fig. 8 B). Adrenaline in doses of 5—20 γ intravenously, which raise the arterial blood pressure considerably in the cat does not change the usual picture obtained on the right side. If it does, the result is a slight decrease in activity. On the left side, however, it causes a certain increase in the electric response of the nerve, particularly when the endings previously were sensitized by veratrine. (Fig. 3 D.)

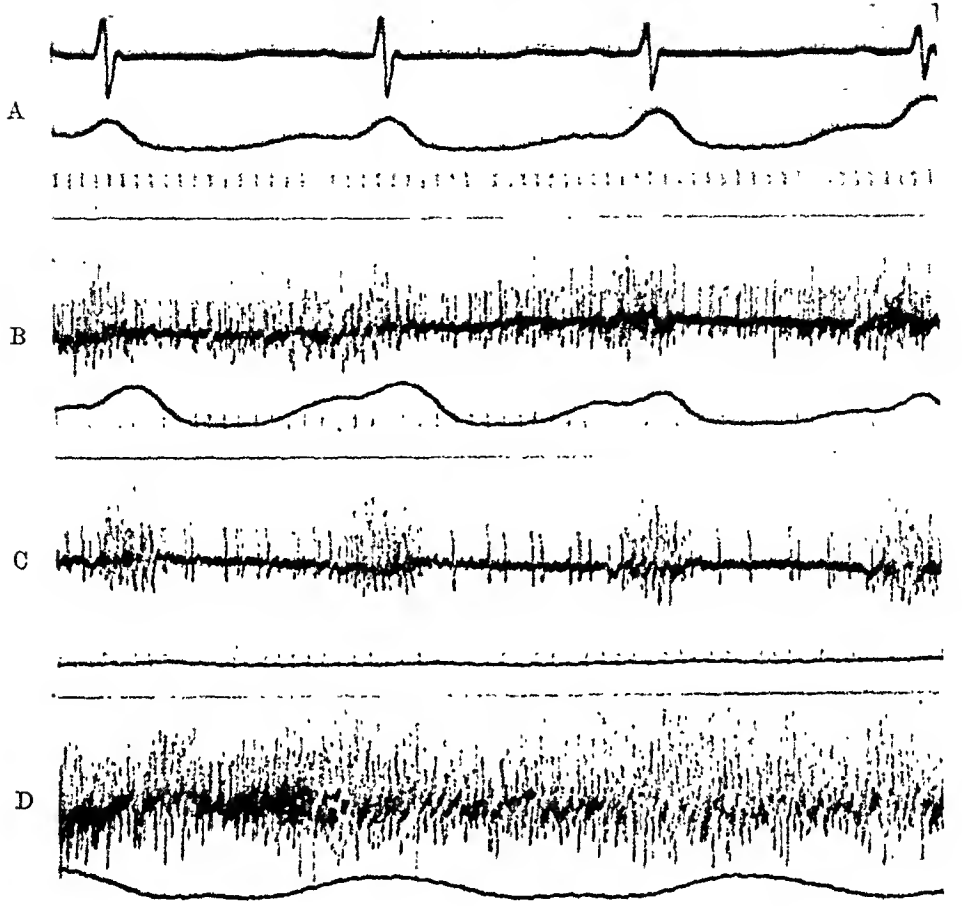

Fig. 5. *A.* ECG and right auricular pressure. *B—D* simultaneous recording of auricular pressure and action potentials from the right side. *B.* Control; *C.* Both caval veins clamped; *D.* After 100 γ veratrine intravenously.

Action of Veratrine. Veratrine, aconitine and mistle-toe extracts produce a very massive continuous discharge of impulses. (Fig. 3 C and 5 D.) The same holds also for adenosin phosphoric acid. (50 mg intravenously). The action of this latter substance came after a latency of about 70 seconds, which olosely corresponds to the latency of the bradycardia caused by this substance first described by DRURY and ST GYÖRGYI (1930).

The effect of veratrine could be abolished very quickly by an intravenous injection of 20—50 mg of $CaCl_2$ as reported by AMANN and SCHAEFER (1943). An injection of 1 cc 2 % sodium citrate enhanced the afferent activity equally distinctly. Thus there can be no doubt that these afferent endings are very susceptible to

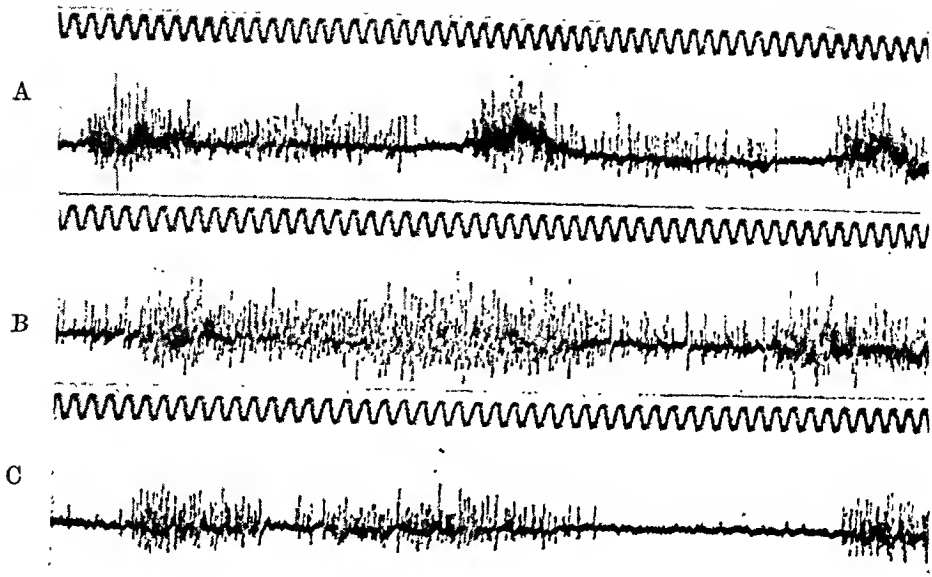

Fig. 6. The afferent impulse traffic in the vagal branch to the *left* auricle *A*. Control; *B*. After clamping the aorta; *C*. Immediately after releasing thea orta.

changes in the calcium concentration of the tissues concerned. We observed a very marked increase in activity 30—60 seconds after the artificial respiration was stopped but it is of course very difficult to decide whether this increase was due directly to the asphyxia or was caused by the accompanying raised activity of the heart and the changed hemodynamic conditions.

The effect of veratrine was also studied on other stretch receptors. Thus we found the veratrine produced a very similar effect upon the carotid sinus nerve, which was promptly abolished by calcium injections. Whether the effect was due to the stimulation of baroceptors or chemical receptors or both has not as yet been properly investigated. In contrast however, the depressor nerve fibres were found not to respond to veratrinc in this manner at doses up to 100 γ, which produced a continuous and very massive outflow of impulses in the afferent heart nerves on both sides.

We have also studied the effect of veratrine solutions 1 : 40,000 dropped directly upon the sternocutaneus muscle of the frog while leading off the action potentials from the nerve. It was found that veratrine here sets up a spontaneous firing of impulses, which however gradually vanishes. The endings were then found to be distinctly sensitized to stretch. Thus loading the muscle with a weight of 7.9 g produced an about 100 % higher frequency of

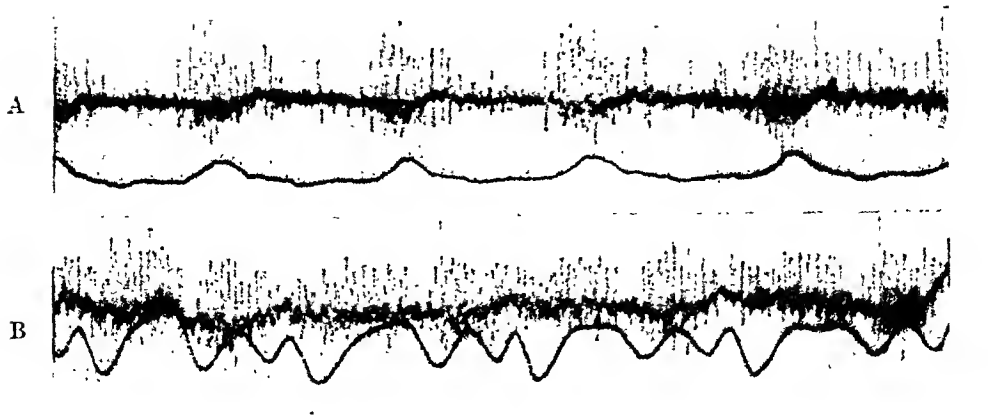

Fig. 7. Auricular pressure and afferent impulses recorded from a very slender vagal branch to the right auricle. *A*. Control; *B*. After clamping the pulmonary artery. Note the small and slowly conducted potentials seen in *A* between the auricular volleys and especially in *B* after clamping of the pulmonary artery.

impulses than before. Later on there appeared another change in the behaviour of the stretch receptors. Normally these receptors are adapting very slowly to a constant stretch as was originally shown by ADRIAN and ZOTTERMAN (1926). Now however these receptors adapt themselves very quickly to the constant stretch, ceasing to fire after a very short interval (see Fig. 9 D). This seems not to be due to any real exhaustion of the receptors causing long recovery as repeated stretchings produced a good effect but must be due to the process of adaptation inherent in the endings. A more detailed study of these phenomen will subsequently be made.

When leading off from very slender nerve strands from the heart slow spike potentials can be seen occurring especially when the intraventricular pressure is raised as for instance during clamping of the pulmonary artery or the aorta. On such occasions however the large spikes produced by afferents from the auricle mask these small and slowly conducted spikes to a very considerable degree. That is of course particularly the case also under veratrine action. But nevertheless the activity of these slow fibres can be traced in the records.

The localization of the afferent nerve endings. The records described and reproduced show that the nervous activity here recorded derives from nerve fibres of class A. Histological examination of the nerve branches concerned show, that there are a few

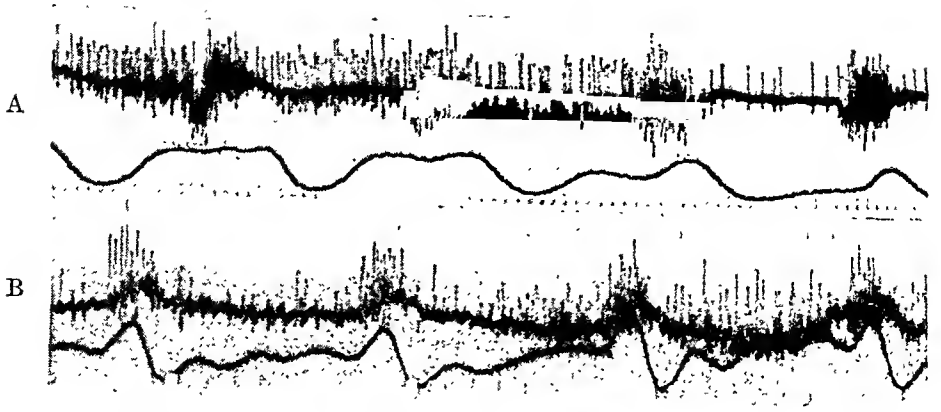

Fig. 8. *A*. The effect of lowering the auricular pressure by clamping the caval veins. *B*. The effect of a sudden injection of 10 cc Ringer in the femoral vein.

fibres of a diameter 2.8—7 μ in diameter. The remaining myelinated fibres are mostly of 1.8—2.8 μ and an abundant mass of fine fibres with very slight or without myeline sheath. Thus the spikes here seen are produced by fairly thin A-fibres. The question now rises where these fibres end in the heart. When leading off from a cutaneous nerve it is generally very easy to obtain a good idea about the receptive field of the nerve in question. Here however the innervated organ is continuously in motion which complicates the task considerably. Now it is very easy to show that there are afferent nerve endings situated in the auricular walls on both sides as a mere touch with a wooden pin on the reigons around and between the caval orifices on the right side and the pulmonary veins on the left side produces a very marked discharges of large spikes. It is very easy to control this by listening to the loud speaker. In order to get further informations on the localization in the heart of these endings we have clamped both caval veins and after cutting a hole in the tip of the auricle we have explored the topography of the endings by means of a wooden pin striking over the auricular structures. In that way we have convinced ourselves that spikes could be obtained by deformation particularly from the orifices of the upper and lower caval vein and from the septum, particularly from a place situated below the foramen ovale.

Touch and light pressure or traction in the ventricles did not produce any spikes of this kind provided that no pull was exerted upon the auricles. In such a case the usual spikes appeared (Fig. 10 A). Thus we must conclude that the spikes here described and

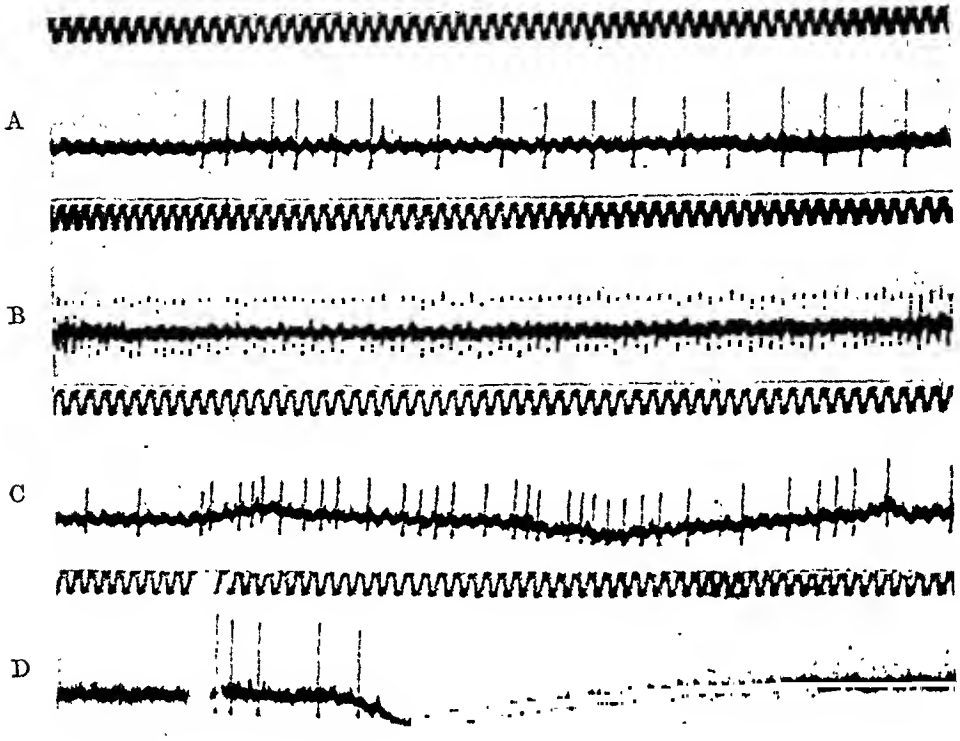

Fig. 9. Records from a sternocutaneous nerve muscle preparation of frog. Single receptor preparation. *A*. Effect of loading the muscle with 7.8 g. *B*. Spontaneous discharge after dropping a veratrine solution 1 : 40,000 on the muscle. *C*. The effect of loading with 7.8 g is considerably augmented after the administration of veratrine. *D*. Later; the same load. Note the rapid adaptation to constant load in D compared to the normal absence of complete adaptation in A. Time 50 cps.

reproduced all belong to myelinated afferent fibres ending in the auricles and the caval veins.

When however the ventricles are handled more roughly, for instance when one pinches the ventricles between the fingers and particularly, when the upper part of the septum is pinched or pressed upon, another kind of response is obtained (see Fig. 10 B, D). We then obtain a response of very slowly conducted spikes easily discriminated from those elicited from the auricles. These slow spikes obviously derive from the ventricles but they can also be obtained by pinching the auricles. These slow potentials no doubt must derive from very thin nerve fibres like those recorded from mesenteric nerves when pinching the intestine (GERNANDT and ZOTTERMAN, 1946).

Electrical stimulation of the afferent fibres. In order to get an

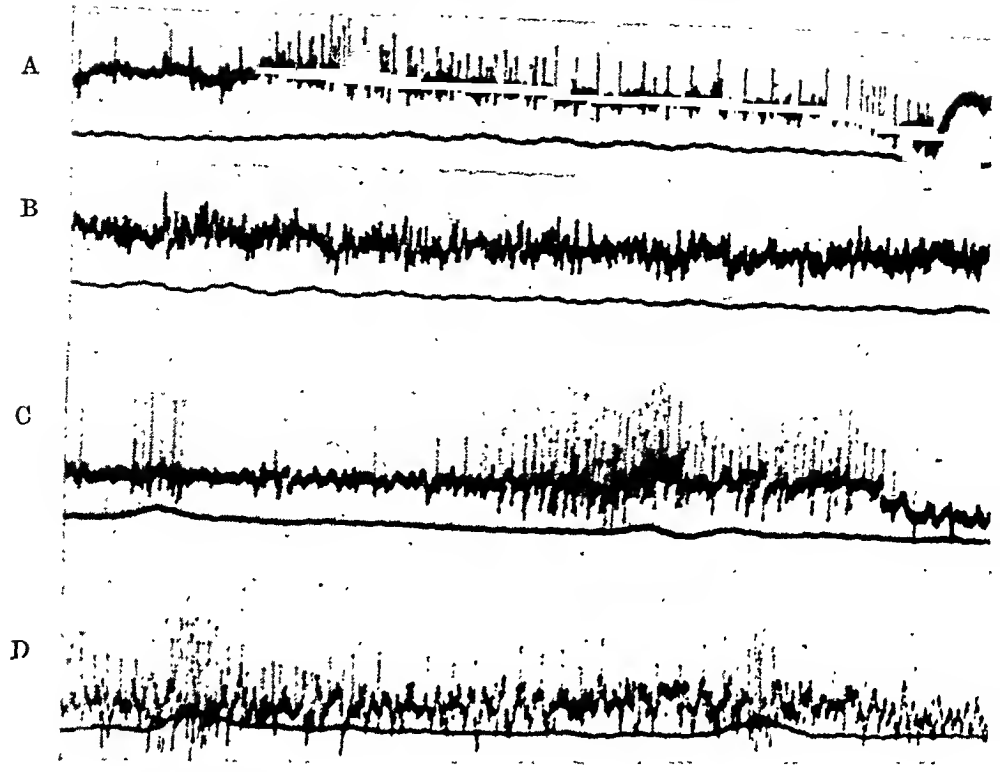

Fig. 10. From a nerve preparation on the right side. *A.* A traction on the auricles is exerted by pulling on the ventricles. Note the numerous relatively large spike potentials. *B.* Pinching the ventricular septum by the fingers. Note the slow and irregular potential changes due to summated small and slowly conducted potentials. *C* and *D* ditto from another preparation. *C.* shows first a volley of large spikes produced by an auricular systole. The next volley was produced by pulling in the auricles from the ventricles. *D.* Pinching the ventricles by the fingers. Note the small and slow potentials which are particularly well seen between the two auricular volleys.

idea about the rôle played by the afferent discharge here recorded we have studied the effect of electric stimulation of the nerve branches concerned upon the arterial blood pressure. In order to obtain a good picture of the heart rate as well as the mean pressure we have recorded the arterial blood pressure by means of a Hg-manometer and a Hürthle-manometer simultaneously. The nerve branches were dissected free until their entrance into the auricles. After ligating these ends with fine silk threads the peripheral ends were cut and all connections to the heart on the operated side were carefully cut in order to avoid any possible direct efferent effect on the heart. The nerve strands could sometimes be obtained for lengths up to 35—40 mm which provided the possibility to secure a stimulation of such a branch without any direct electric spread of the stimulation to the vagus

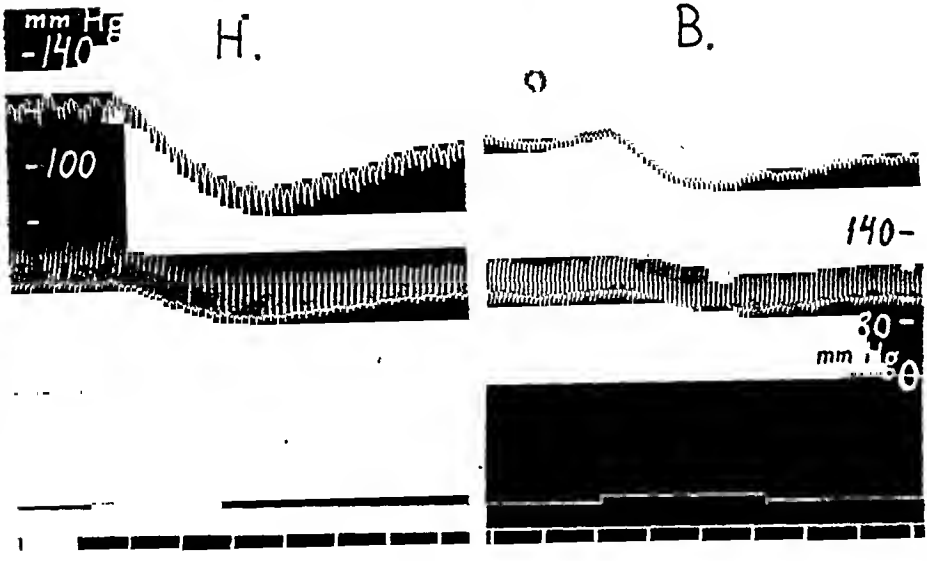

Fig. 11. Cat 2.8 kg. Chloralose-urethane anestesia. Blood pressure recorded by Hg-manometer (upper tracing) and Hürthle manometer. Time 4 sec. *A*. Central end of upper vagal branch to right auricle stimulated by rectangular pulses, 2 volts, 80 cps. The vagus stem was cut below the heart and all vagal branches to the right lung and heart were cut. *B*. The same stimulation applied to the central end of the vagal nerve branch running to the *left* auricle.

stem. For the stimulation we have used rectangular pulses of varying frequency, duration and amplitude.

Whenever an effect on the blood pressure was produced by electrical stimulation a fall in pressure always followed. On a few occasions only this fall was proceeded by a short and very slight increase in pressure in late stages of the experiments. Any acceleration of the heart was never observed. This holds for both sides. In fig. 11 it can be seen that stimulation on the right side with 1 volt slowed the heart rate and lowered pressure. The same stimulation applied on the left side did not produce any effect on the heart rate, as the right vagus was cut, but it produced a definite fall in blood pressure. The threshold for stimulation was very distinct. It was very considerably higher than for the phrenic nerve which generally reacted at a strength which was only a few per cent of that necessary to bring about a depressor reflex from these nerve branches running to the heart. A maximum was generally obtained by 2 volts, frequency 80 cycles. When the frequency was reduced to low values the response was diminished and no effect at all could then be obtained by increasing the

voltage. When in some stages a slight initial rise in blood pressure was observed we could never find that this rise could be separated from the accompanying strong fall in blood pressure by any change in the stimulation. Thus variation in the duration of the shocks, their frequency or strength did not produce any change in the order of events. In other words in such cases we could not find any means of separating the slight rise from the accompanying distinct depression. An important fact is that the reflex fall in blood pressure was equally well produced from the left side as from the right side. It is further very important that we are sure that we can exclude any direct stimulation of the heart itself, its efferent fibres or afferent fibres originating in the walls of the aorta on the right side, where the aortic depressor nerve sets off higher up at the root of the subclavian artery. On the left side however NONIDEZ (1935) has described afferent fibres ending in the central parts of the aorta, "rami aortici vagi" which run in the vagal branches to heart here experimented upon. Such fibres may be excited by the electric stimulation. Their activity is however not seen in our records of the afferent impulses as in those experiments all fibres but those which run across the pulmonary artery towards the left auricle were carefully severed. That this is the fact is demonstrated by the records themselves.

Discussion.

The adequate stimulation. The afferent impulses here recorded are obviously discharged by mechanical means. They appear to derive from stretch receptors and we can therefore expect them to display a general behaviour of the kind previously described by ADRIAN and ZOTTERMAN (1926) and MATTHEWS (1933). Our experience of these endings in the auricles show that they are easily excited by very slight traction. It is thus very obvious that these receptors must be excited by the movements of heart when these lead to stretching of the tissues. Further any filling of the heart which stretches these structures would produce the same result. Thus the persistence of the auricular volley after clamping both caval veins when as the records show the venous pulse disappears, must be due to a stretching of the receptors situated around the orifices of the caval veins by the contraction of the auricles. Our mechanical scanning of the auricles has shown these regions to be particularly sensitive to deformation and NONIDEZ (1935, 1941)

PANNIER (1940) and DE PALUMBI and VERGA (1941) found in these regions a richness of afferent nerve endings which are very similar to those described by DE CASTRO in the walls of the carotid sinus and the aorta at the depressor region. NONIDEZ has also, as far as his description goes, followed the nerve fibres from these endings up in the nerve branches to the vagus, from which we have led off our impulses. These findings agree very well with the experiment of AMANN and SCHAEFER (1943). But when these authors maintain that the ventricle synchronous volley originate at least partly from endings in the ventricles we cannot follow them. When applying direct mechanical stimuli we could never elicit any of these spikes when the stimulation was restricted to the ventricle and when any pulling of the auricle was excluded. Thus the relatively rapidly conducted spikes recorded by these authors as well as by us cannot derive from any other region than from the auricles, the caval vein and the pulmonary veins.

By this we do not exclude the discharge of afferent impulses from the ventricles themselves, but we wish to emphasize that the ventricular impulses are conducted in thinner nerve fibres which give rise to spikes of quite another appearance on the records (see Fig. 10). These small slow spikes are very easy to distinguish from the more rapidly conducted spikes originating entirely from the auricular regions. Such slow impulses were traced after veratrine poisoning also by AMANN and SCHAEFER (1943), who suggested that they were pain fibres. The normal discharge of impulses occurring besides the auricular volley here recorded and previously described by these authors consist however principally under normal conditions only of rapid spikes. Even in our most slender nerve preparations where the signal-to-noise ratio was most favourable as in Fig. 4 only speedy and big spikes are to be seen. When the ventricles however are more distended as after clamping the aorta or the pulmonary artery there appear signs of small spikes. During veratrine poisoning the big spikes increase in such an order that the smaller spikes are masked nearly entirely. There are however definite signs that these small fibres are excited by strong distension of the ventricular walls (Figs. 6 B and 7 B). These volleys of slow impulses do not seem to be a phenomenon of such a normal occurrence as are the rapid impulses from the auricles but constituting a defense mechanism against undue distension of the ventricles. It is of course very likely that these small fibres may give rise to pain

when sufficiently stimulated and we might look upon the marked fall in arterial blood pressure brought about by veratrine — the "Bezold effect" — as a kind of a nociceptive reflex.

The Bainbridge reflex. The relatively large spikes here reproduced and previously described by AMANN and SCHAEFER and recently by WHITTERIDGE originate from endings in the auricles. These endings are very sensitive to stretch and they would no doubt constitute a very suitable receptor mechanism for the Bainbridge reflex. It would thus judging from our records be very tempting to suggest that the system of very sensitive endings in the auricles subserved the Bainbridge reflex, while the system of slow fibres from the ventricles elicited the oppositely acting Bezold effect. The electrical stimulation of the nerve branches concerned has however hitherto not given much support to such a view. We have never seen any acceleration of the heart following upon any stimulation of the fibres in question. Further we have not seen any acceleration when a body-warm Ringer solution was forcibly injected into the femoral vein on the otherwise intact cat. On the contrary we have always found that such an injection produces a slowing of the heart rate. The arterial blood pressure may rise at first to fall afterwards. Sometimes there is no initial rise. The fall in arterial pressure however is the constant phenomenon.

Microscopic examination of the nerve branches running to the right auricle show that they contain altogether a very low number of myelinated fibres as seen by Table I.

Table I.

Number	of	fibres	7 —5.6 μ — 2
»	»	»	5.6—4.2 μ — 10
»	»	»	4.2—2.8 μ — 15
»	»	»	2.8—1.4 μ c:a 50
»	»	»	$<$1.4 μ any great number.

We have reason to assume that the rapid spikes originating from the auricles refer to myelinated fibres of a diameter above 2.8 μ. Their total number should thus not exceed 30 fibres. Among the smaller fibres we have to count with a certain number of preganglionic vagal and postganglionic sy mpathetic fibres. The number of very thin afferent fibres is however very much greater than the number of larger afferent fibres even when we suppose that only a third of the total number of thin fibres should be

afferent fibres. The total number of the very small afferent fibres subserving the Bezold effect from the ventricles should thus be quite sufficient to account for the very powerful depressor reflex, when these fibres are sufficiently stimulated. The very high threshold to electrical stimulation also fits in very well with this assumption.

If the larger fibres were responsible for the Bainbridge acceleration we should expect to obtain such an effect when using stimulation below threshold. In spite of the very high and sharp threshold of the depressor effect which would favour such a discrimination by stimulating means we have not been able to find the slightest trace of any acceleration — only the opposite effect.

We must therefore conclude that we have not been able as yet to find any facts which support the assumption of a heart accelerating reflex elicited by afferent impulses from the right auricles, although afferent nerve endings, who might subserve such a reflex, can easily be demonstrated. These receptors seem to have a lower mechanical threshold and the threshold to electrical stimulation of their nerve fibres would also according to their larger diameters be considerably lower than that of the thinner fibres.

Summary.

1. The afferent impulse traffic of the vagal nerve branches running to the heart on both sides has been studied by means of recording the action potentials from the nerves, simultaneously with the electrocardiogram or the pressure changes in the auricles.

2. Spike potentials from fibres of a diameter 2.8—7 μ are elicited from a limited number of endings around the orifices of the caval veins and from the auricular septum of the right side as well as around the orifices of the pulmonary veins in the left auricle. These receptors are stimulated by stretching caused by distention of the auricle by increased pressure and by the mechanical events of the heart.

3. Besides the larger spike potentials originating from the auricles, very small spikes can be seen, which obviously are produced by very thin nerve fibres. These are strongly stimulated by clamping the pulmonary artery or the aorta and by veratrine and mistle-toe extracts. Very massive volleys of very low spike height and of slow conduction can be seen isolated when pinching the

ventricles. They display a general character very similar to those recorded when noxious stimuli are applied to the skin and the intestine in such a way that no large fibres are stimulated.

4. The afferent heart nerve fibres responsible for a depressor reflex present a very high threshold to electric stimulation thus indicating their character of thin fibres (δ_2 or C-fibres). The depressor reflex elicited from the heart is thus produced by the activity of very thin afferent fibres ending in the auricles and the ventricles. Whether the larger afferent fibres ending exclusively in the auricular regions on both sides produce any separate reflex activity is still under investigation.

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tinuously the pulse frequency. The experimental set-up is schematically reproduced in fig. 1. The rebreathing system consists of a KROGH-spirometer with a capacity of 7 litres, a soda-lime absorber, and a large ordinary bell-spirometer (capacity 150 litres). An electrical pump ensures complete mixing of the air.

Before starting the experiment the apparatus is washed out with atmospheric air, and the spirometer bell is raised, usually to 30 litres. Hence the total volume of the apparatus becomes 50 litres. (Estimated through mixing with a known amount of hydrogen and subsequent analysis.)

The spirometer bell has been furnished with a pawl-device, through which it is possible in a few seconds to lower the bell exactly 5 litres. Aside from its use as a means of thus lowering the total volume of the apparatus, this spirometer acts as a reservoir and remains fixed throughout the experiment.

At the start of the experiment the KROGH-spirometer is empty, but as soon as the subject is connected to the apparatus 5 litres of atmospheric air is passed into it from the bell-spirometer. Thus the starting volumes of the apparatus are always identical. Each time the KROGH-spirometer is emptied during the experiment, it is refilled with 5 litres of air from the bell spirometer (applying the pawl-device).

Experimental Course.

During some preliminary investigations the respiratory response was found to be more distinct with the subject working than with the subject at rest. Hence it was decided to let the subject perform a slight work (200 kgm per minute) on KROGH's bicycle ergometer (1913) during the experiment. Moreover working has shown to be helpful in diverting the subjects attention from his respiration, which thus becomes less restrained.

The pulse frequency is registered with JOHNSEN's pulse time recorder (1945), the respiration traced as usual by the KROGH-spirometer, and the minute volume of the respiration with a modified TYBJÆRG HANSEN ventilation measurer (1945).

The arrangement described has the drawback, that the oxygen tension is decreasing directly from the start of the experiment. As it was soon found that the changes in pulse and respiration of several subjects appeared very rapidly, the procedure was slightly altered in the later experiments. A starting period was

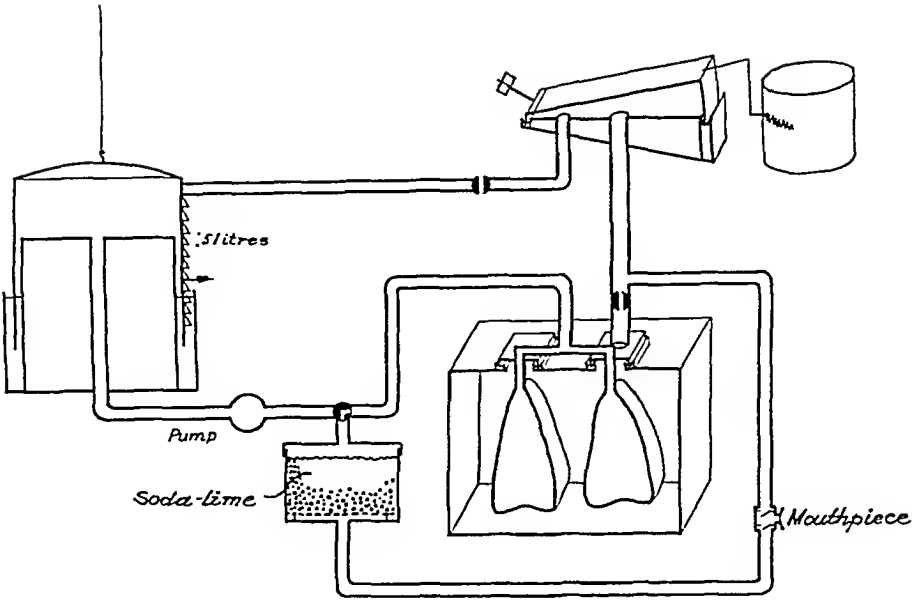

Fig. 1. Schematical representation of the experimental arrangement. With the cocks turned as shown in the figure the subject is breathing atmospheric air, inspired from the box in the middle of the figure and expired through the soda-lime to the two Douglas-bags inside the box. When all three cocks are turned 90°, the subject is switched into the rebreathing circuit.

introduced, in which the subject is breathing atmospheric air for about 5 minutes before the rebreathing period. This was obtained by adding to the initial set-up an apparatus previously described (SONNE and GEORG 1945), the box and bags shown in fig. 1.

In this case the inspiratory air is drawn from the box, which is filled with atmospheric air, and the subject expires into the rubber bags. When the steady state values have been registered in this way, the subject is switched on to the rebreathing circuit (cf. fig. 1). The transfer, which is imperceptible to the subject, is preferably made at a moment when the KROGH-spirometer is just empty.

Toward the end of the experiment, oxygen is usually conveyed from a reservoir of 20 litres pure oxygen, which is shunted into the circuit. (This is not shown at the figure.)

Results.

Fig. 2 reproduces a typical kymographic tracing of an experiment with the latter experimental arrangement. The pulse is registered at the top, JOHNSEN's apparatus recording as ordinates

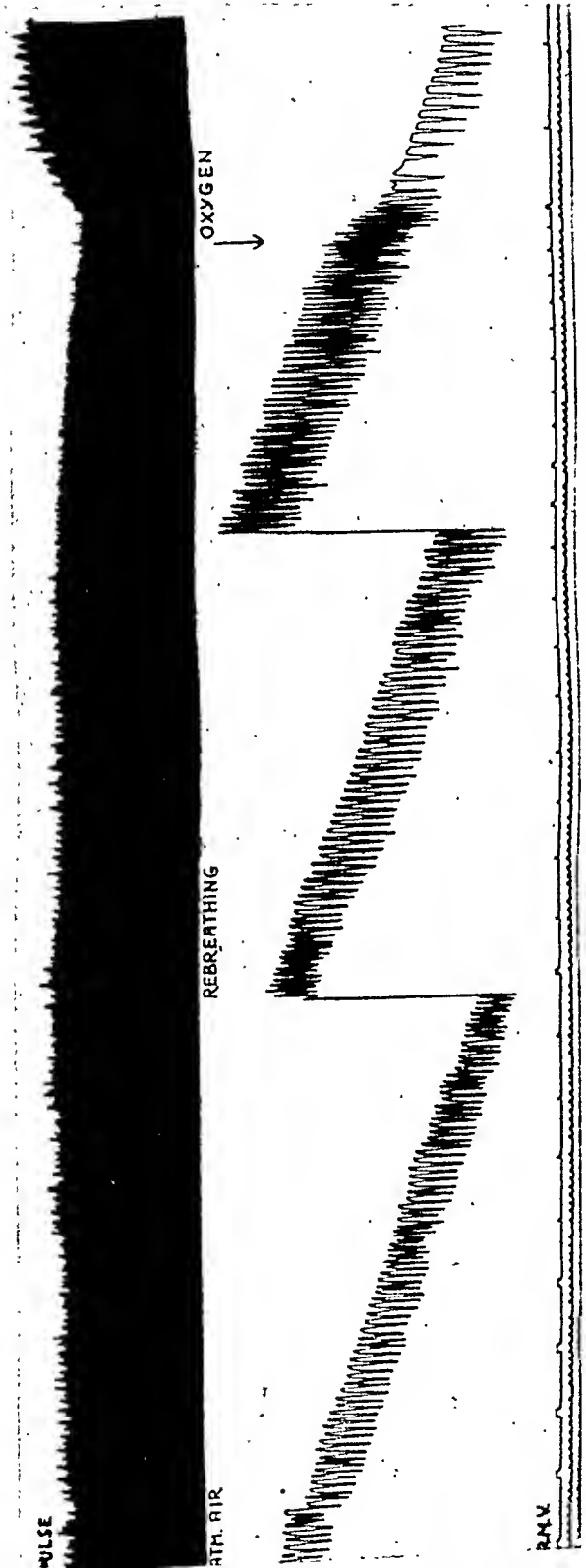

Fig. 2. Rebreathing experiment on normal subject. (For details see text.)

Table I.

Rebreathing Experiments, Starting in Atmospheric Air, Initial Volume of the System 50 Litres.

Subject	Minute-volume of respiration (litres)											
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	minute
L. M. S. 2—2	14	13	12	13	13	14	15	17	19	21	23	
» 5—2	14	16	15	16	17	18	22	24	28			
» 26—3	11	13	13	12	15	16	16	18				
» 11—4	13	12	13	14	14	15	17	18	20	23	27	
» 12—4	16	16	12	15	16	17	18	21	22			
» 25—4	12	13	12	15	15	15	16	17				
S. J. 26—3	7	10	12	11	12	14	13	15	15	17		
» 12—4	8	11	11	15	17	18	15	18				
» 13—4	12	12	12	11	14	16	16	25				
J. G. 2—2	15	15	15	15	16	17	22	25				
» 5—2	16	17	17	17	18	21	26	30				
» 7—3	16	14	15	17	18	22	24					
» 8—3	14	15	15	16	20	22						
» 3—4	12	13	15	15	18	17	20	23	30			
» 26—4	12	13	13	16	17	18	20	22	24			

the time interval between the pulse beats. (This is obtained by elevating the recording lever with a constant rate, each pulse beat momentarily lowering it to the starting position.) The undulations of the curve represent the respiratory arrhythmia, which is waning with increasing pulse frequency (*i. e.* with decreasing ordinates of the pulse time recorder).

Below this is registered the respiratory tracing, the declination of which represents the oxygen consumption as usual. Throughout the first period the subject respires in atmospheric air, during the two next he is rebreathing. In the middle of the last period oxygen is conveyed, followed — as can be seen — by a momentary decrease in pulse frequency and ventilation.

At the bottom of the kymogram time-marking is represented, being in tenths of a minute with every tenth stroke left out. Directly above, the curve of the ventilation measurer is seen, every ten litres being marked by a jag.

It is to be seen from the tracing, that the increase in pulse frequency occurs rather imperceptibly, slight at the start and more pronounced later. It is very difficult to point out when the change of frequency is starting. (In the case shown the pulse frequency was increased from 104 to 124 per minute.)

Exactly the same applies to the ventilation. The respiratory volume per minute, drawn from the curve of the ventilation measurer, is represented in table I—II. All subjects are normal.

Table II.

Rebreathing Experiments as in Table I, but Preceded by a 5-minute Period in Atmospheric Air.

Subject	Minute-volume of respiration (litres).														
	atm. air					rebreathing									
						1.	2.	3.	4.	5.	6.	7.	8.	9.	10. minut
J. G. 3—5	12	13	13	13	13	11	12	14	14	15	18	19	20	22	
» 14—5	12	12	12	13	11	13	13	15	15	16	18	18	23	26	
» 14—5	13	12	13	14	13	15	15	16	16	17	18	21	27	32	
C. L. 22—5	11	14	14	15	15	16	18	19	21	22	23				
H. J. S. 24—5	13	14	13	13	14	13	14	14	17	16	16	18	17		
C. J. 25—5	18	18	17	18	18	19	19	19	19	20	20	20	21	23	26
K. H. H. 19—7		14	14	14	15	15	16	16	16	17	19	21	24		

Table I gives the values from the initial experimental course, whereas table II represents the experiments initiated by a period of breathing unchanging atmospheric air. The respiratory volumes are given in whole litres only. Owing to the variability found normally, even where the external conditions are equal, a greater accuracy is not sought for.

In all subjects the ventilation is seen to increase gradually. Usually it is possible to indicate, however, a minute in which a significant increase seems to be occurring. This juncture varies from the first to the sixth minute. The duration of the experiments averages 9 minutes, the oxygen consumption being on the average 7 litres or about 780 ml per minute. Thus it follows that the average oxygen percentage in the inspiratory air at the end of one minute is 19.7 %, and at the end of six minutes is 12.9 %.

The final oxygen percentage has been measured in 26 experiments, the values found between 7.1 and 12.2 %. (These figures are higher than those found by Hohwü Christensen and Krogh (1936), but our subjects have not endeavoured to continue as long as possible; moreover, these investigations are made during work, those of Hohwü Christensen and Krogh in rest.)

Thus it is found, that the oxygen tension, where a noticeable compensatory response occurs, is varying not only from subject to subject, but also in the same individual at different times. Furthermore: as the compensatory reactions in some normal subjects are perceivable already at an oxygen content of 19—20 % in the inspiratory air, it is obvious that in this way a differentiation between normal and pathological lung function is not fea-

sible. (This may be done by starting the experiment in air with a higher oxygen content than the atmospheric air, *e. g.* 25—30 %.)

The question which arises next is, whether this variability found is due to different ability of the subjects to maintain the arterial oxygen tension despite decreasing alveolar tension, or if it is caused by a varying sensitivity of the chemoreceptors toward decreased arterial oxygen tension.

In an attempt to elucidate this, we have supplemented the experiments described with a set of experiments in which the arterial oxygen tension is followed simultaneously with an oximeter (MILLIKAN 1942). An experiment typical of these is reproduced in fig. 3.

The upper curve represents the arterial oxygen saturation, the other curves follow the description given to fig. 2. The deflections of the galvanometer in the oximeter set-up are registered photographically, the time-marking being in common with the other parts of the apparatus. Hence it is possible to draw this curve in relation with the others.

It is seen that the arterial oxygen tension decreases already in the first or second minute, before any change in pulse or ventilation is observable. (Samples of inspiratory air taken during this experiment contained: at the end of one minute: 19.7 %, at the end of two minutes: 18.5 % oxygen.)

These experiments have lasted 5—6 minutes only (*i. e.* until an oxygen percentage in the inspiratory air of about 13—14), and have all been interrupted before the subject has experienced any discomfort. Repeated experiments on this and other normal subjects have given similar results.

Comment.

Judging from these experiments, it seems that in normal subjects (performing a slight work) even a very small decrease in the oxygen tension of the inspiratory air suffices to lower the arterial oxygen tension. In some subjects this is followed almost simultaneously by a compensatory reaction (viz. increase in ventilation and pulse frequency), whereas in other subjects a greater decrease in arterial oxygen tension seems necessary before any response in pulse or ventilation is noticeable.

v. EULER, LILJESTRAND and ZOTTERMAN (1940) suggested, on the basis of animal experiments, that the chemoreceptors are

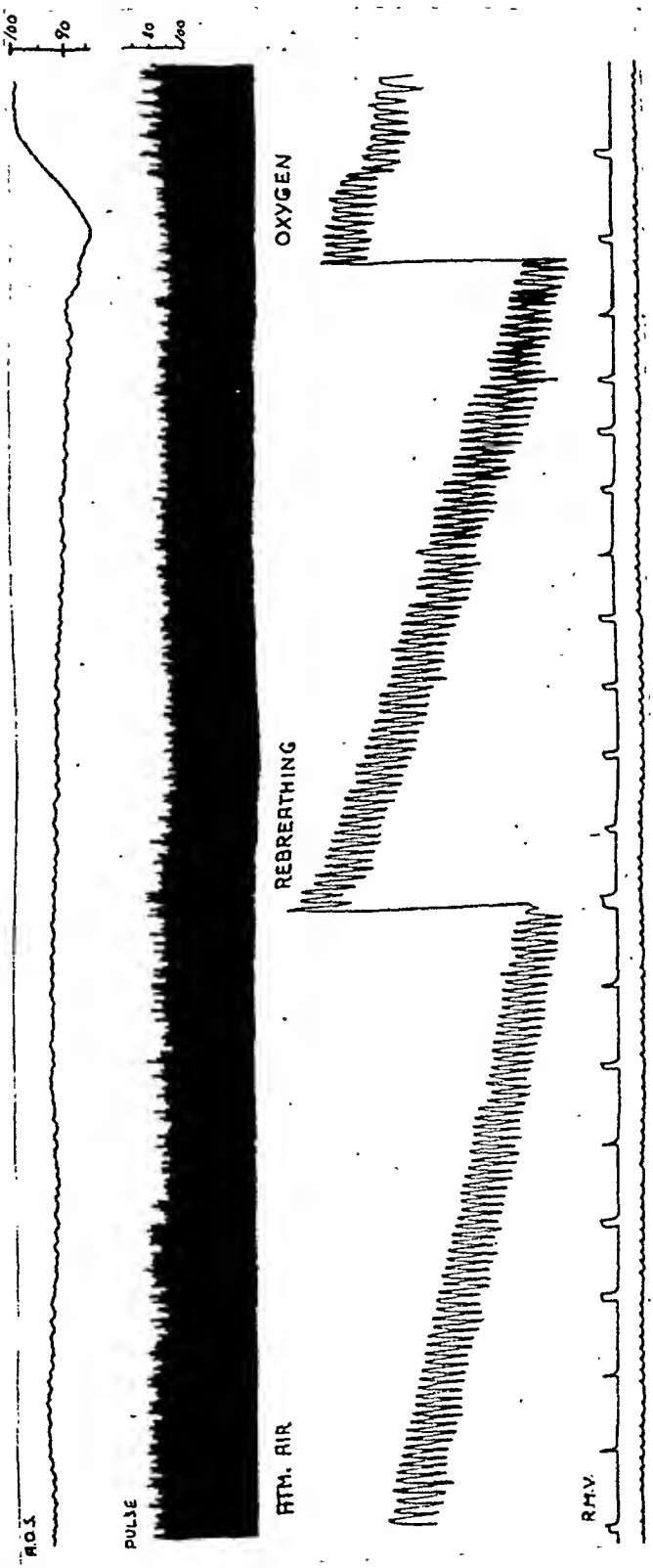

Fig. 3. Rebreathing experiment on normal subject, with continuous photoelectrical recording of the arterial oxygen saturation.

tonically active in man also. The very early chemoreceptor response found in some of our experiments indicates that this may hold true in some subjects. In others such an assumption does not seem warranted. It must be kept in mind, however, that a difference in reaction does not necessarily mean a difference in chemoreceptor response, but may as well be due to a varying reaction of the respiratory centre toward the chemoreceptor impulses.

The rather moderate increase in respiratory minute volume should not lead to any hasty conclusion as to the strength of hypoxemia as a respiratory stimulant. It must be remembered that the hypoxemic stimulus is counteracted by a decreasing alveolar carbon dioxide tension and a shift in blood pH toward alkalinity. This is evident from the apnea following the abolition of hypoxemia, when oxygen is given at the end of the experiment. Several of the symptoms experienced during hypoxemia may be due to hypocapnia.

Summary.

Rebreathing experiments have been performed on normal subjects during a light work (200 kgm per min.). The respiration, respiratory minute volume and pulse frequency are registered continuously. In some later experiments the arterial oxygen saturation is recorded too, employing a MILLIKAN-oximeter.

It is found that ventilation and pulse frequency are changing gradually during the experiment, a noticeable change first occurring at an oxygen percentage of between 20 and 13 % in the inspiratory air.

The oxygen percentage at which a noticeable change occurs, is varying not only from subject to subject but also in the same subject at different times.

A decrease in arterial oxygen tension usually seems observable earlier and more regularly than any change in pulse frequency or ventilation. Thus it seems that the varying response of different individuals toward decreased oxygen tension in the inspiratory air is largely due to differences in the sensitivity of the chemoreceptors (or the respiratory centre), and not to any different quality of lung function.

Addendum: After the conclusion of these experiments there has appeared an important paper by DRIPPS and COMROE (Am. J. Physiol. 1947. *149.* 277) dealing with this subject. These authors,

carrying out their investigations on normal persons during rest, state that an increase in pulse frequency usually is observable before any change in ventilation. From our tracings such a difference is not clearly visible, otherwise our findings seem in accordance with those of DRIPPS and COMROE.

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Identification of the Sympathomimetic Ergone in Adrenergic Nerves of Cattle (Sympathin N) with Laevo-Noradrenaline.

By

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Certain evidence has previously been presented to show that the neurohormone of adrenergic cattle nerves differs from adrenaline but closely agrees with its non-N-methylated homologue, arterenol or noradrenaline (EULER, 1946, a, b). BACQ, who was the first to suggest in 1934 that the excitatory sympathin E of CANNON and ROSENBLUETH is identical with noradrenaline, has recently, with FISCHER (1947), given further support to this concept. In the present paper some experiments are reported which produce additional evidence for the identification of the sympathomimetic neurohormone in splenic nerves of cattle with noradrenaline and also support the probable assumption that the active principle occurs in the laevoform.

Methods.

A. Preparation of extracts. In addition to the previously used method of extraction with 2 volumes of ethanol to which 1.25 ml. 10-N H_2SO_4 was added per litre, extractions were also made with 10 per cent trichloracetic acid. In the latter method the fresh splenic nerves were carefully freed from their sheath, finely cut with scissors and extracted over night in the ice chest. After filtering, the trichloracetic acid was removed with ether. With simple laking of the whole nerves in trichloracetic acid the extraction was less rapid and complete

but had the advantage of giving almost colourless extracts. The extracts were adjusted to pH 3.5—4 and kept in the refrigerator.

B. Test methods.

Cat's blood pressure. The cats were anaesthetized with 6 c. c. 1 p. c. chloralose per kg. i. v. In order to stabilize the blood pressure level 0.05—0.1 mg ergotamine tartrate was given i. v. (EULER and SCHMITERLÖW, 1944). Atropine sulphate was given 1 mg. per kg. subcutaneously. The histamine effects of the extracts were excluded with antergan[1] 5 mg. per kg. intramuscularly and 1 mg. per kg. i. v. Cocaine hydrochloride 6 mg. per kg. was given i. m. in some cases. Finally dibenamine was used in a dose of 5 mg. per kg. i. v. in order to discriminate between adrenaline and nor-adrenaline, the action of the former being reversed and that of the latter only diminished. The effects of the extracts were matched against dl- or l-noradrenaline kindly supplied by dr. M. L. TAINTER.

Hen's rectal caecum. The rectal caecum of the fowl was suspended in a bath with Tyrode's solution containing half the usual amount of potassium, as recommended by BARSOUM and GADDUM (1935).

Cat's uterus in situ

The movements of the uterus were recorded from the free end of one of the horns of the organ in situ after separation from the endometrium.

C. Colorimetric determination. The method used was that of EULER (1933) with some modifications. To 0.3—1 c. c. of the extracts, previously adjusted to pH 5—6, was added 0.3 c. c. of a buffer solution consisting of 1 c. c. 2-NH_2SO_4 and 6 c. c. 20 % NaAc. Iodine solution, 0.1-N was added to a yellow colour. After 5 minutes the excess was removed with 0.1-N $Na_2S_2O_3$ and water added to 2 c. c. and the colour read in a colorimeter against dl- or l-noradrenaline hydrochloride. It was found advantageous to use a yellowish background in order to minimize the influence of disturbing colorations which sometimes occurred.

D. The substances used for comparison were l-adrenaline base dissolved in hydrochloric acid, dl-noradrenaline hydrochloride and l-noradrenaline hydrochloride.

Results.

I. Colorimetric determinations.

The resolution of the synthetic racemic noradrenaline into its optical isomers recently accomplished by TULLAR has provided a new means of getting nearer to the exact chemical nature of the noradrenaline-like factor studied. Like l-adrenaline the laevoform of noradrenaline has about twice the activity of the

[1] A sample of this substance (Lergitin) was kindly given to me by A. B. Recip, Stockholm.

racemic product (TAINTER, TULLAR and LUDUENA, 1948) as has been confirmed in the present study.

A comparison of the amounts of catechol substances in terms of dl- and l-noradrenaline in splenic nerve extracts determined biologically on the cat's blood pressure and colorimetrically gave the following results.

Table I.

(Figures in μg.)

Splenic nerve extracts	Biological activity in terms of		Colour corresponding to		Ratio	
	dl-noradrenaline HCl	l-noradrenaline HCl	dl-noradrenaline HCl	l-noradrenaline HCl	Color. Biol. dl-noradr.	Color. Biol. l-noradr.
I	100		55		0.55	
II	70		40		0.57	
III ...	94		53		0.56	
IV	63		40		0.63	
V	100	54	60	60	0.60	1.1
VI		36		35		0.97

As seen in the table, the ratio between the colorimetrically and the biologically determined values is about 0.6 for dl-noradrenaline, whereas it is closer to 1 for l-noradrenaline. Since there might be certain errors in the biological as well as in the colorimetrical determinations (owing to the appearance of some slightly disturbing coloured products in the extracts on addition of iodine) the figures are only approximate but nevertheless the results seem to warrant the conclusion that the active substance in the extracts consists of l-noradrenaline. Even a moderate admixture of l-adrenaline would raise the ratio considerably since the biological activity of l-adrenaline is, on an average, $^1/_4$ of that of l-noradrenaline on the cat's blood pressure under the existing experimental conditions (cf. p. 009). Thus even if only 10 p. c. of a biological activity corresponding to 100 μg. l-noradrenaline were due to adrenaline in the extracts of splenic nerves one would obtain:

Biological activity	Colorimetrically determined amount
90 μg. l-noradrenaline	90 μg. l-noradrenaline
40 μg. l-adrenaline	[1]44 μg. »
100 μg. l-noradrenaline	134 μg. l-noradrenaline.

[1] Approximately, since there is a slight difference in colour.

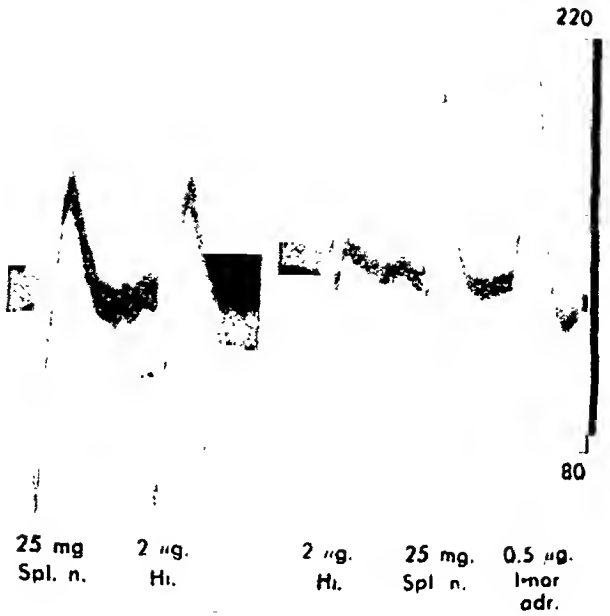

Fig. 1. Cat. Chloralose. Blood-pressure records. Atropine. Effect of 25 mg. splenic nerves from cattle and 2 µg histamine, before and after antergan 6 mg. per kg. Time ½ min.

A ratio of 1.34 would thus be obtained but such high figures have not been observed. The absence of a corresponding fluoreseence reaction also speaks against an admixture of adrenaline to higher amounts than a few per cent. The same implication ean be drawn from the results of biological evaluation on the fowl's reetal caecum.

II. Cat's blood pressure.

The good agreement between the biologieal aetion of purified extracts of splenic nerves and noradrenaline on the cat's blood pressure before and after cocaine, and also after ergotamine and dihydroergotamine has previously been demonstrated (EULER, 1946 a, b). Owing to the presence of contaminating depressor substances, a biological analysis could not hitherto be made with non-purified extracts. Again, since it might be argued that the purification procedures modify the action of the extracts in various ways, apart from the fact that quantitative determinations become hazardous, it would be desirable to find some way of neutralizing the biological impurities. A closer study of the depressor activity of the splenic nerve extracts revealed that

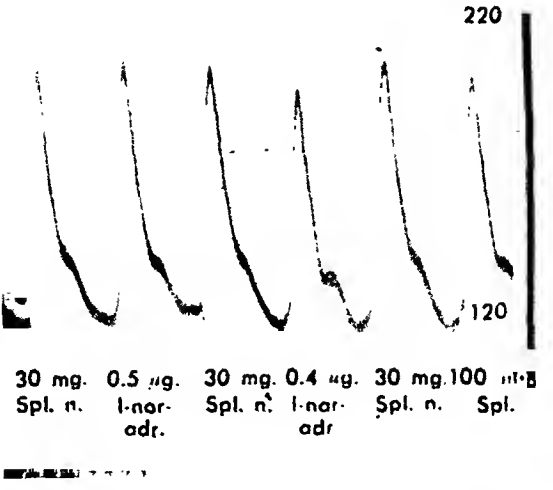

Fig. 2. Cat. Chloralose. Blood-pressure records. Atropine, antergan.
(1), (3), (5) Extract of 30 mg. splenic nerves of cattle.
(5) 0.5 µg. l-noradrenaline.
(4) 0.4 µg. l-noradrenaline.
(6) Extract of 100 mg. spleen of cattle.
Time ½ min.

it was caused predominantly by unexpected large amounts of histamine (EULER, 1948 a). There are reasons to believe that the high histamine content has caused misinterpretations of some of our earlier results with regard to the estimated content of adrenaline-like activity in crude nerve extracts, owing to the liberation of adrenaline from the adrenals (cf. SCHMITERLÖW, 1948).

By the introduction of antihistaminic compounds (HALPERN, 1942) a convenient means of excluding histamine effects of organ extracts was placed at our disposal. Fig. 1 shows the effect of a crude splenic nerve extract on the blood pressure before and after antergan. The pure pressor effect after antergan could be matched with noradrenaline permitting in most cases a satisfactory determination. The remaining pressor effect was destroyed by treating the extract with iodine at a pH above 7 or heating it in the presence of $FeCl_3$ (0.2 mg. in 1 c. c.) at pH 8. Fig. 2. illustrates a direct comparison of an unpurified splenic nerve extract and l-noradrenaline, which has been used as a standard in the later experiments in accordance with the findings reported above with the colorimetric method.

As a further aid to the analysis of the action, sympathicolytic compounds were used in order to discriminate between adrenaline

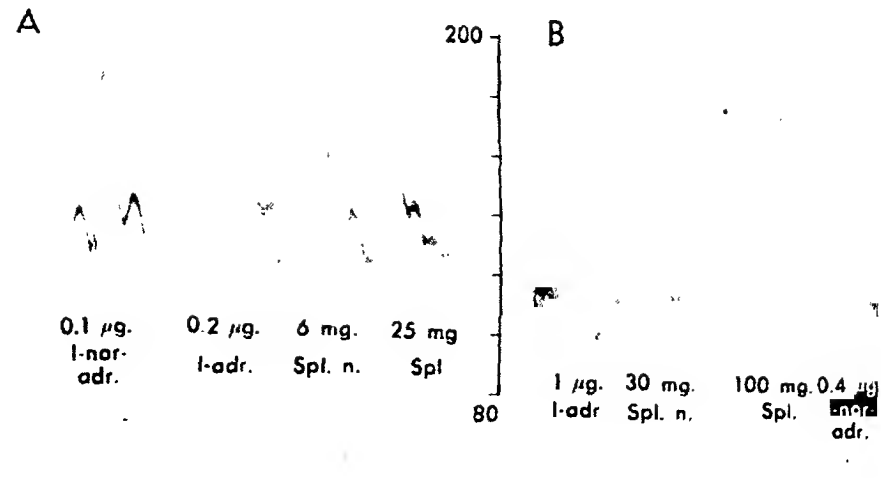

Fig. 3. Cat. Chloralose. Blood-pressure records. Atropine, antergan, cocaine.
A. Before dibenamine. (1) 0.1 µg. l-noradrenaline. (2) 0.2 µg. l-adrenaline. (3) Extract of 6 mg. peeled splenic nerves of cattle. (4) Extract of 25 mg. spleen of cattle.
B. After 5 mg. dibenamine per kg. (1) 1 µg. l-adrenaline. (2) Extract of 30 mg. splenic nerves of cattle. (3) Extract of 100 mg. spleen of cattle. (4) 0.4 µg. l-noradrenaline.
Time ½ min.

and noradrenaline. Dihydrocrgotamine proved quite useful for this purpose in previous experiments by causing a reversal of the adrenaline pressor effect in the cat, whereas the action of noradrenaline and cattle nerve extracts were diminished but not reversed. In the present work dibenzyl-dichloroethylamine (dibenamine) (NICKERSON and GOODMAN, 1947) has been used and found advantageous since it produces analogous effects with great regularity and without side actions. Fig. 3 shows the enhanced effects of splenic nerve extracts and l-noradrenaline after cocaine, and the reversal of the adrenaline effect but not that of the nerve extract or l-noradrenaline after dibenamine.

III. Fowl's rectal caecum.

GADDUM and BARSOUM discovered in 1935 that the rectal caecum of the hen was extremely sensitive to adrenaline. A comparison of the relative sensitivity of this preparation to adrenaline and noradrenaline revealed a sensitivity ratio of about 10: 1 to 25: 1 for l-adrenaline and l-noradrenaline, a fact of great significance for the discrimination of the two substances. The fowl's rectal caecum thus compares favourably with some of the test preparations indicated by WEST (1947) for a similar purpose.

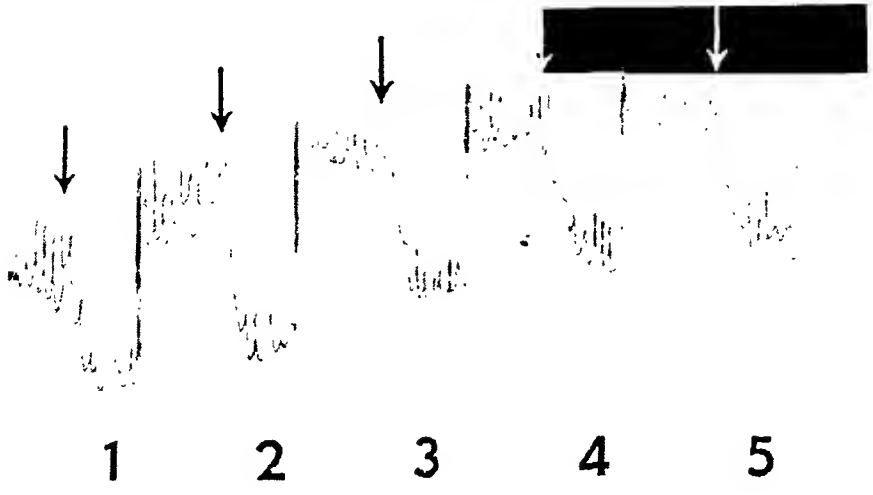

Fig. 4. Fowl's rectal caecum, suspended in Tyrode's solution, containing $^1/_2$ the usual amount K+.
(1), (3), (5) 0.036 μg. l-adrenaline.
(2) 2.2 μg. dl-noradrenaline.
(4) 1.5 μg. dl-noradrenaline.

The ratio l-adrenaline: dl-noradrenaline was determined to 50: 1 in the experiment illustrated by fig. 4.

Fig. 5 shows a comparison of the effects of an extract of splenic nerves, l-adrenaline and l-noradrenaline on the hen's rectal caecum. The biological effect on the cat's blood pressure in this particular extract equalled 14 μg. l-noradrenaline per g. On the preparation used for the experiment in fig. 5 the activity of the splenic extract corresponded roughly to 15 μg. l-noradrenaline per g. It is also evident that an amount of 0.1 μg. l-adrenaline elicited a stronger action than 100 mg. splenic nerves, the blood pressure action of which, in terms of adrenaline, would amount to more than 5 μg. From this it follows that less than 2 p. c. of the activity could be due to adrenaline.

The probability that another catechol derivate would fit in so closely with the biological activity of the nerve extracts on the cat's blood pressure after cocaine and on the fowl's rectal caecum as l-noradrenaline is indeed very remote, considering the ratios of activity, being in one case 0.25: 1 and in the other about 25: 1, or roughly 100 times as high in one case as in the other.

IV. Cat's uterus in situ.

The agreement between the action of splenic nerve extracts and of l-noradrenaline is obvious from the tracings in fig. 6.

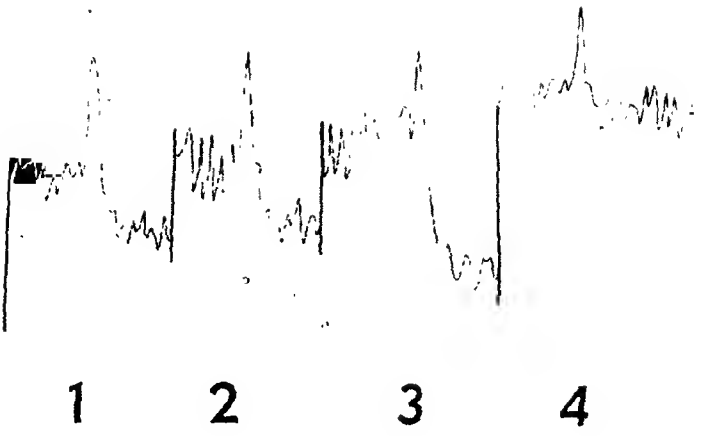

Fig. 5. Fowl's rectal caecum, suspended in Tyrode's solution with $^1/_2$ the usual amount K+.

(1) Extract of 100 mg. splenic nerves of cattle.
(2) Same as (1) but heated $^1/_2$ min. at pH 8 with 0.2 mg. $FeCl_3$ per c. c., neutralized, and 1.5 μg. l-noradrenaline added.
(3) Same as (2) but instead of l-noradrenaline 0.1 μg. l-adrenaline added.
(4) Same as (2) without additions.

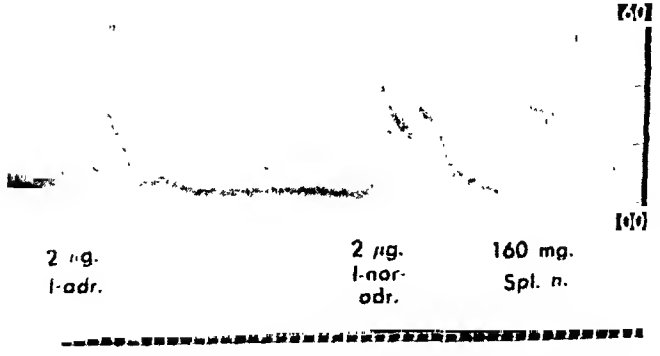

Fig. 6. Cat. Chloralose. Upper curve: Movements of uterus horn in situ. Lower curve: Blood pressure, atropine, antergan.

(1) 2 μg. l-adrenaline.
(2) 2 μg. l-noradrenaline.
(3) Extract of 160 mg. splenic nerves of cattle.
Time $^1/_2$ min.

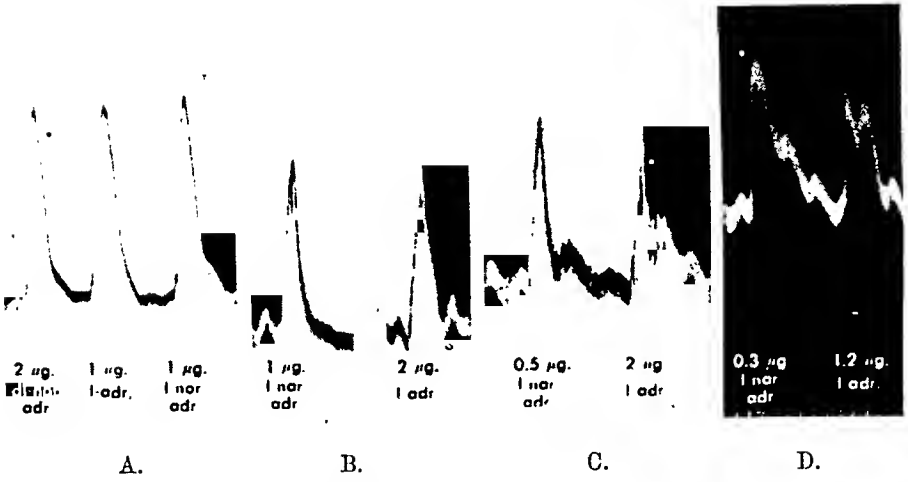

Fig. 7. Cat. Chloralose. Blood-pressure records from 4 different animals.
A. (1) 2 µg. dl-noradrenaline.
(2) 1 µg. l-adrenaline.
(3) 1 µg. l-noradrenaline.
B. (1) 1 µg. l-noradrenaline.
(2) 2 µg. l-adrenaline.
C. (1) 0.5 µg. l-noradrenaline.
(2) 2 µg. l-adrenaline.
D. (1) 0.3 µg. l-noradrenaline.
(2) 1.2 µg. l-adrenaline.
Time ½ min.

V. Quantitative evaluation of sympathomimetic activity in extracts of splenic nerves and spleen.

In the previous sections of this paper evidence has been brought forward to show that the amount of adrenaline in the splenic nerve extracts does not exceed a few p. c. of the total activity or total amount of catechol compounds. This is also in agreement with the figures given by RAAB (1943) for the specific ratio of the colour reaction of SHAW with extracts of spleen, giving no indication of adrenaline.

Even though several tests tend to show the presence of minor amounts of adrenaline it would seem permissible for practical purpose to evaluate the total activity as observed on the blood pressure of the cat in terms of l-noradrenaline, which has shown such a good agreement with the active factor that identity seems to be beyond any reasonable doubt. On this basis the content of fresh splenic nerves of cattle (without sheath) has been determined to 12—16 µg. l-noradrenaline hydrochloride per g.

The amount of sympathomimetic activity in alcoholic extracts of spleen has previously been estimated to about 10 µg. l-adre-

naline per g. Fig. 2 permits an approximate evaluation of the content of spleen of cattle when extracted with trichloracetic acid to about 4 μg. l-noradrenaline per g. which can be regarded as roughly equivalent to the above mentioned value. It should be noted, also, that owing to the large variations in the activity ratio between adrenaline and noradrenaline (Fig. 7) an exact estimate of cattle nerve sympathin should not be made in terms of adrenaline.

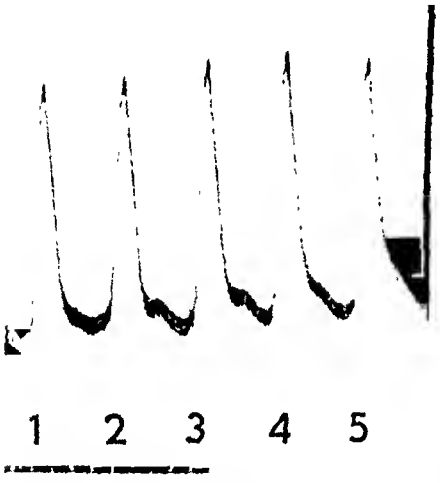

Fig. 8. Cat. Chloralose. Blood-pressure records.
(1), (3), (5) 1 μg. dl-noradrenaline.
(2) 0.5 μg. l-noradrenaline.
(4) 0.6 μg. l-noradrenaline.
Time ½ min.

On the other hand there would be no serious objection against giving the estimates in terms of dl-noradrenaline instead of l-noradrenaline, since these compounds show a constant ratio of about 0.5: 1 (Fig. 8). It seems more logical, however, to give the values as l-noradrenaline, since this substance is most likely the naturally occurring one and is available.

Since the relation between the content of sympathin in splenic nerves and in the spleen itself is roughly 1: 4, this must mean that either the amount present in the nerve fibres in the splenic stroma is very much higher than that of the nerves running in the stalk, or that sympathin occurs outside the nerve fibres in the spleen. Which is true cannot be decided at present.

Comment.

In their recent paper BACQ and FISCHER (1947) suggest that the term sympathin should be used to denote any mixture of noradrenaline and adrenaline that might be liberated from adrenergic nerves. This suggestion implies that adrenergic nerves might contain and liberate noradrenaline as well as adrenaline in varying proportions in one and the same animal. If adrenaline is present besides noradrenaline in extracts of organs, the occurrence of chromaffine cells in the extracted tissue should always be con-

sidered, however. It may be recalled that the prostate gland, which contains a substance behaving like adrenaline biologically and colorimetrically, also contains numerous groups of chromaffine cells (EULER, 1934). Nor can the presence of such cells be excluded in nerves of sympathetic origin. Viewing the extensive quantitative and, on certain occasions, even qualitative differences in action between noradrenaline and adrenaline it seems less probable that the organs for their functions should depend on the liberation of a mixture of these two substances, in varying proportions, perhaps according to the outcome of some methylation process. Before it has been demonstrated that both ergones occur in the axones of one nerve it seems safer to assume that the activity is due to l-noradrenaline present in the axones, and that the adrenaline, if present simultaneously, is derived from scattered chromaffine cells. This does not exclude the possibility that the adrenergic transmitter in certain nerves is adrenaline, as indeed the experiments of GADDUM and KWIATKOWSKI (1938, 1939) and GADDUM, JANG and KWIATKOWSKI (1939) on rabbit nerves indicate.

If the term sympathin of CANNON and BACQ should be maintained, but in the meaning of the true mediator of adrenergic nerves as suggested by EULER (1946 b) — and recently supported by BACQ and FISCHER (1947) — it seems necessary, however, to introduce some distinction in order to avoid confusion, since more than one mediator may come into play. With regard to the present knowledge of the nature of the mediators it would seem most adequate to use the terms Sympathin N (for noradrenaline) and Sympathin A (for adrenaline) (EULER, 1948 b).

Summary.

The sympathomimetic substance in extracts of splenic nerves from cattle closely agrees with l-noradrenaline colorimetrically (iodine method) and biologically.

The amount of the active substance in splenic nerves without sheath in terms of l-noradrenaline is about 10—15 μg. per g. and for whole spleen 2—4 μg. per g.

From the colorimetrical and biological tests it appears that the adrenaline content of the splenic nerve extracts does not exceed 0.5 μg. per g., corresponding to less than 2 p. c. of the total activity on the blood pressure.

The relative activity of l-noradrenaline and dl-noradrenaline is roughly 2: 1 as measured on the blood pressure of the cat.

The relative activity of l-noradrenaline and l-adrenaline on the blood pressure of the cat varies from 1: 1 to 5: 1, and on the fowl's rectal caecum from 1: 10 to 1: 25.

It is suggested that the term sympathin N should be used to denominate the adrenergic nerve transmitter having the properties of noradrenaline.

The discrepancies often observed between adrenolytic and sympaticolytic action of certain drugs may be explained on the ground that the sympathetic transmitter in these cases is noradrenaline, which is considerably more resistant to the blocking action of *e. g.* dibenamine than adrenaline.

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Studies on the Circulation in Man.
II.

Normal Values for Cardiac Output and Pressure in the Right Auricle, Right Ventricle and Pulmonary Artery.

By

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with the technical assistance of ELSA EKBERG and HANS ANDUREN.

Received 31 March 1948.

The cardiac output has already been determined with the direct Fick method in a considerable number of normal persons (2, 7, 8, 9). Reports on the normal pressures in the right heart and the pulmonary artery are still limited.This study aims to compare our results with the Fick method with the results of earlier investigations and to extend the knowledge about the pressures in the lesser circulation.

Almost all earlier pressure recordings are made with the Hamilton manometer. We have used the capacitive manometer according to Tybjærg-Hansen and Warburg. In contrast to the Hamilton manometer this is critically damped. The technical details of our procedure have been reported earlier (6).

The material consists of healthy medical students and patients without circulatory disturbances and in perfect general condition. Most of them were referred to the hospital for observation during which no objective signs of illness were found.

Pressures.

Fig. 1 shows examples of normal pressure tracings from the right ventricle and auricle and brachial artery, fig. 2 from the brachial and pulmonary arteries. The data concerning the pres-

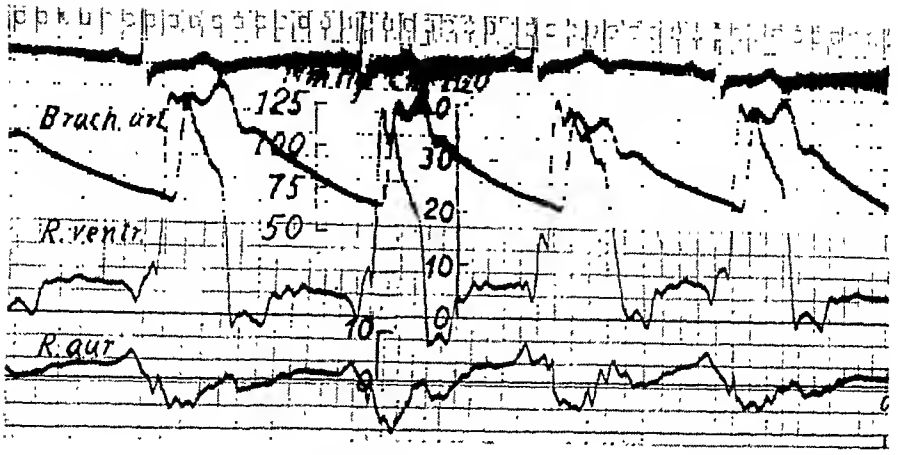

Fig. 1. Simultaneous tracing of ecg, brach. art., right ventricle and right auricle in a normal woman.

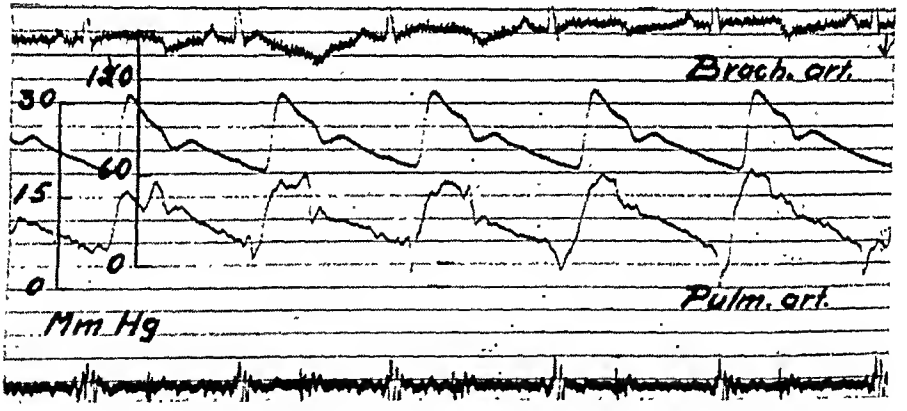

Fig. 2. Simultaneous tracings of ecg, phonocardiogram and pressures in the brachial and pulmonary arteries on a normal man.

sures in 13 of the persons, whose cardiac output was considered basal are compiled in table 1. The pressures in the systemic arteries and in the right heart and the pulmonary artery varied considerably. As could be expected the systolic pressure in the pulmonary artery and the right ventricle were about the same and higher in cases with a slow heart rate than in cases with fast. The same applies to the pulse pressure. The systolic pressure in the right ventricle and the pulmonary artery never exceeded 30 mm of mercury.

Table 1.

Blood Pressures in mm Hg in the Brachial Artery, Pulmonary Artery, Right Ventricle and Right Auricle in 13 Cases with Normal Circulation.

Case	Brachial Artery			Pulmonary Artery			Right Ventricle			R.Aur.
	Syst.	Diast.	Mean	Syst.	Diast.	Mean	Syst.	Diast.	Mean	Mean
C. Å. H.	121	74	90	29	8	19	30	3	13	—
I. L.	143	89	113	14	4	9	20	1	4	1
R. K.	126	72	96	—	—	12	28	7	10	5
K. Y. S.	106	68	87	24	11	16	26	4	10	2
N. H.	151	86	110	—	—	—	19	4	9	5
B. L.	120	77	95	18	5	11	24	2	6	1
B. L.	120	77	97	19	7	12	19	3	6	—
K. G. E.	150	95	118	26	8	18	26	3	9	1.5
I. M. B.	—	—	—	22	8	15	22	3	10	1
S.	151	85	112	26	11	18	26	5	12	11
G. R.	160	92	121	27	9	16	30	2	10	—
G. S.	128	80	99	19	10	15	17	6	11	3
B. H.	112	60	88	24	9	15	24	8	11	4
Average	132	80	100	23	8	15	24	4	9	2.75

Table 2 shows the pressure figures in three cases in which the cardiac output was probably not basal. All of them fall within normal limits.

In table 3 and 4 the data concerning the pulmonary artery pressure and right ventricular pressure are summarised and compared with the results of earlier investigations. There is an excellent agreement between the different reports.

Cardiac Output.

The Oxygen consumption, A—V differences, cardiac output, pulse rate, cardiac index and stroke volume in 13 determinations in 13 individuals are tabulated in table 5 together with the calculated oxygen consumption/sqm BSA. The determined oxygen consumption exceeded the calculated by more than 15 % in some cases. The pulse rate in other cases was more than 80 or the arterial blood pressure more than 140 systolic. When only one of these symptoms appeared separately the individual was considered basal. The cardiac index did not show any systematic relation to these symptoms. Repeated determinations showed good agreement. The differences in the double determinations were due mainly to changes in oxygen consumption and not to changes in A—V differences.

Table 2.

Blood Pressures in mm Hg in 3 Cases with Normal Circulation with Elevated Cardiac Output.

Case	Brachial Artery			Pulmonary Artery			Right Ventricle			R.Aur.
	Syst.	Diast.	Mean	Syst.	Diast.	Mean	Syst.	Diast.	Mean	Mean
C. E. L.	142	67	96	25	9	16	24	5	10	—
F. N. K.	138	80	103	25	9	16	26	7	12	—
D. L.	137	97	114	14	5	8	18	1	7	0
Average	139	81	104	21	8	13	23	4	10	0

Table 3.

Normal Pressure in the Pulmonary Artery (in mm Hg).

Investigator	Nr Cases	Systolic		Diastolic		Mean	
		Average	Range	Average	Range	Average	Range
Cournand 1947 .	4	25	—	8	—	15	—
Werkö 1947	7	19	14—24	5.8	3.4—9	11.3	8.8—13.3
Dexter et al 1947	3	26	20—32	10	8—12	—	
Lagerlöf-Werkö 1948.....	11	23	14—29	8	4—11	15	9—19

Table 4.

Normal Right Ventricle Pressure (in mm Hg).

Investigator	Nr Cases	Systolic		Diastolic		Mean	
		Average	Range	Average	Range	Average	Range
Bloomfield et al 1946.....	17	25	18—30	2.5	0.5—4.5	—	—
Werkö 1947	14	25	16—34	3.1	0.5—7.2	8.4	4.4—11.9
Dexter et al 1947	3	26	20—32	3.7	0—6	—	—
Lagerlöf, Werkö 1948.....	13	24	17—30	4	1—8	9	4—13

In table 6 the average value for the oxygen intake, oxygen A—V difference, cardiac otuput and cardiac index are compiled with the results of earlier investigations. Though the cardiac output and cardiac index in the present investigations on an average are somewhat higher than in earlier reports there is no significant difference.

In table 7 the data from 3 individuals who were not considered basal, is tabulated. The cardiac index is high only in one of the cases though the oxygen consumption has considerably increased.

Table 5.

Cardiac Output and Vascular Resistance in 13 Cases with Normal Circulation.

Case	Age years	Body Surface Area m^2	Pulse Rate per min.	Oxygen consumption			A—V oxygen diff. cc/l	Cardiac Output			Vascular resistance dynes sec cm^{-5}	
				cc/min.	cc/min. per m^2 BSA	Predicted cc/min per m^2 BSA		cc/min	cc/min per m^2 BSA	per beat cc.	peripheral	pulmonary[1]
C. A. H.	36	1.82	80	268	147	128	34	7,880	4,330	108	882	162
I. L.	32	1.52	85	220	145	121	44	5,000	3,280	63	1,790	128
R. K.	26	1.78	54	211	119	119	36	5,850	3,290	108	1,217	68
K. Y. S.....	32	1.92	87	240	125	131	37	6,500	3,380	74	1,020	148
N. H.	24	1.94	74	268	138	133	33	8,120	4,180	110	1,040	—
B. L.	25	1.61	85	210	131	122	30	7,000	4,350	85	1,060	103
B. L.	24	1.82	75	282	155	134	39	7,200	3,980	96	1,035	122
K. G. E.....	38	1.66	80	249	150	125	34	7,300	4,400	92	1,260	164
I. M. B.....	23	1.45	79	185	128	125	32	5,780	3,980	73	—	166
S.	24	1.90	61	234	123	135	36	6,500	3,420	106	1,310	160
G. R.	23	1.90	62	235	124	136	38	6,180	3,250	100	1,540	181
G. S.	24	1.94	70	265	137	133	43	6,150	3,180	99	1,205	117
B. H.	29	1.78	64	240	135	119	37	6,500	3,650	102	984	86
Average.....	27	1.77	74	239	135	128	36	6,620	3,740	94	1,190	134

[1] Calculated from the mean pressure difference between the pulmonary artery and the right auricle with the assumption, that the right auricular pressure does not differ much from that of the left auricle. Investigations in progress during the proof reading indicate, that the normal left auricular pressure is some millimeters of mercury higher than the right auricular pressure.

Table 6.

Cardiac Output. Normal Values.

Investigator	Nr Cases	Oxygen cons cc/min. Average	Oxygen A—V Diff cc/l. Average	C. O. l/min. Average	C. I. l/min/m² BSA Average
Cournand et al 1945.....	34	245	45	5.5	3.3
Mc Michael, Sharpey-Schafer 1944	—	—	—	5.3	3.3
Stead et al 1945	18	245	40	6.2	2.8—4.0
Rasmussen 1946	—	—	—	—	3.6
Werkö 1947 ...	14	253	42	6.3	3.7
Lagerlöf, Werkö 1948 .	13	239	36	6.6	

Table 7.

Cardiac Output in 3 Cases with Normal Circulation and Increased Oxygen Consumption.

Case	Pulse Rate /min.	Oxygen Consumption			A—V O_2 diff cc/l	Cardiac Output		
		cc /min.	cc/min. per m² BSA	Predicted cc/min. /m² BSA		cc /min.	cc/min per m² BSA	cc /beat.
C. E. L.	71	346	181	123	52	6,650	3,500	94
F. N. K.	75	325	184	127	52	6,240	3,500	83
D. L.	98	258	141	120	24	10,800	5,900	110

All blood samples of mixed venous blood in the present study are taken from the pulmonary artery, where the mixing of blood is complete. Most of the earlier published values for cardiac output are calculated from oxygen content in blood samples from the right auricle. Though the blood usually is well mixed in the auricle in normal subjects, sometimes a difference between the oxygen content of the auricular and the ventricular blood has been demonstrated.

Vascular Resistance.

The average peripheral vascular resistance in this study was found to be 1190 dynes cm sec^{-5} with a range from 882 to 1 790 dynes cm sec^{-5}. The pulmonary vascular resistance was 134 dynes cm sec^{-5} with a range from 68 to 181 dynes cm sec^{-5}. These figures are in accordance with those earlier published.

Discussion.

This study confirms the pressure values set forth by the Bellevue group for normal right auricular, ventricular and pulmonary artery pressure (1). This is of interest as the earlier values are determined with the Hamilton manometer and the present values with a critically dampened electrical manometer. It seems safe to conclude from this and earlier studies that the normal systolic pressure in the right ventricle and the pulmonary artery during basal conditions never exceeds 30 mm of mercury and rarely is below 20 mm Hg. The end-diastolic pressure in the right ventricle may normally amount to 8 mm, but is usually considerably less, about 2—3 mm Hg. The diastolic pressure in the pulmonary artery is more difficult to determine exactly, due to the oscillations in the pressure curve at the end of diastole. In altogether 25 published cases it has varied between 3 and 11 mm of mercury. The left auricular pressure at the end of diastole should be lower than the end-diastolic pressure in the pulmonary artery.

The values for cardiac index is in accordance with those earlier found using the same technique. They are considerably higher than those found using the Grollman acetylene inhalation technique. This has been claimed to be due to the physical irritation the catheterization induces in the test subjects. Work now in progress shows that the same difference in the cardiac output values obtained with the two methods also exists when the methods are used simultaneously on the same subject. It is thought that the difference is due to a recirculation of blood during the period of acetylene equalisation, making the Grollman values too small (11).

There was no increase in the pressures in the lesser circulation in three cases with elevated oxygen consumption and increased cardiac output. This is in accordance with the data of Hickam and Cargill, who did not find any change of the pulmonary artery mean pressure in normal individuals when they did a limited work that doubled the oxygen consumption and the cardiac output (5).

Summary.

1. The cardiac output has been determined in 13 normal individuals using the direct Fick principle after catheterization of the pulmonary artery.

2. The average value for cardiac output was 6.6 l/min or 3.7 l/min/m² BSA.

3. The pressures in the brachial artery, pulmonary artery, right ventricle and right auricle were recorded in the same individuals using a critically damped electrical manometer.

4. The average pressures were: in the brachial artery 132/80, mean 100 mm Hg, in the pulmonary artery 23/8, mean 15 mm Hg, in the right ventricle 24/4, mean 9 mm Hg, in the right auricle mean 2.8 mm Hg.

5. The results are compared to earlier published.

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The Source of the Histaminolytic Enzyme in the Blood of Pregnant Women.

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The presence of histaminase during pregnancy has engaged the interest of many observers (MARCOU et al., 1938, ZELLER, BIRKHÄUSER, MISLIN and WENK, 1939, EFFKEMANN and WERLE, 1940, ANREP, BARSOUM, IBRAHIM and AMIN, 1941, AHLMARK, 1944, ANREP, BARSOUM and IBRAHIM, 1947, and others). The most important contribution to this subject has been made by AHLMARK (1944). In this paper he called attention to the diagnostic and prognostic value of estimations of the histaminase content of the plasma carried out at an early stage of pregnancy.

EFFKEMANN and WERLE (1940) demonstrated the presence of the histaminase enzyme in the placenta. They held, however, that it could not be carried over into the maternal blood. ANREP, BARSOUM and IBRAHIM (1947) studied apart from the histaminolytic action of the placenta also that of the blood from the umbilical cord and of the amniotic fluid. These authors used as a measure of the histaminolytic action the histaminolytic index "HI" suggested by MARCOU (1938). This index represents the percentage of histamine destroyed in 30 minutes at 37° C by blood to which 3 γ histamine diphosphate had been added per ml. They observed that in blood collected from pregnant women the "HI" began appreciably to increase from about the end of the second month of a 9-months-pregnancy and continued to increase throughout the period of gestation from 0 % to 92 %. According to ANREP, BARSOUM and IBRAHIM, there was no evidence of a histaminolytic action in blood specimens collected at an earlier stage of pregnancy. Neither did they detect any such action in blood from non-pregnant women. In blood from the

umbilical cord they found that the "HI" ranged between 25 % and 33 % whereas that of the maternal blood was approximately 95 %. In the opinion of these authors, the weak histaminolytic action of the foetal blood argues against the assumption that the foetus is the source of the histaminolytic agent, particularly in view of the fact that, after parturition, the histaminase disappears more rapidly in the child than in the mother. They wrote that, calculated per g. of tissue, the histaminolytic action of the placenta was 10 to 15 times stronger than that calculated per ml. of a 9-months-pregnancy serum. They did not observe any significant difference in the histamine inactivation by placental extracts, neither early in pregnancy nor at full term. Neither did they detect any histaminolytic action in the amniotic fluid nor in extracts from the umbilical cord. ANREP, BARSOUM and IBRAHIM felt that they have brought forward evidence that the placenta is the source of the histaminolytic agent found in the maternal blood. In 1941, ANREP, BARSOUM, IBRAHIM and AMIN published some cases in which pregnancy resulted in the intrauterine death of the foetus, and where the "HI" of the maternal blood had rapidly decreased. On the basis of these observations these authors arrived at the conclusion that "HI" estimations offered the possibility of ascertaining whether the foetus had died.

AHLMARK, in his paper stated above, discussed also the relation between the histaminolytic actions of the plasma and the placenta. According to his observations the difference between the histaminolytic action of the plasma and that of the placenta is somewhat greater than that reported by ANREP, BARSOUM and IBRAHIM. AHLMARK (1944, 1947) observed that in blood collected from pregnant women the histaminolytic action began rapidly to increase in approximately the seventh week of pregnancy and continued to increase noticeably during the first half of gestation reaching values which were 5,000 times higher than those observed in non-pregnant women; later, until parturition, the values remained approximately at the same level.

Personal Investigations.

In the present study interest was mainly focussed on the estimations of the histaminolytic action of blood from the umbilical cord, placenta, maternal blood and of the amniotic fluid. In some

cases these investigations were extended to the foetal blood, foetal parenchymatous organs, umbilical cord, ovaries and so forth, and also to blood collected from healthy men and non-pregnant women. In addition, a few cases were studied in which pregnancy resulted in the intrauterine death of the foetus.

The estimations of the histaminolytic action were made by the methods elaborated by AHLMARK (1944). The principles of these methods are briefly as follows:

After the addition of a known amount of histamine to the fluid whose histaminolytic power is to be estimated, the mixture is incubated for a definite time at a constant temperature. The fluid is then chemically treated and the remaining amount of histamine determined by assaying the sample on the surviving ileum of the guinea-pig against a standard solution of histamine. The histaminolytic power is expressed by the amount of histamine diphosphate that is inactivated by 1 ml. fluid or 1 g. tissue in one hour under known standardized conditions (γ/ml./hour or γ/g./hour).

The methods used in collecting the specimens will be described in greater detail because during the course of the investigations it became evident that the results depend largely on the manner in which the specimens are collected. In addition, attention will be directed to sources of error which may arise.

In all the cases here reported maternal blood was collected by puncturing a cubital vein with a dry needle after having cleansed the skin with ether. We avoided cleansing the skin with alcohol because alcohol, even applied in very small quantities, may interfere with the biological tests. Approximately 50 ml. blood were collected in a small flask containing a few drops of tricresol-free heparine-solution. After centrifugation of the mixture the plasma was decanted and its histaminolytic power estimated.

Blood from the umbilical cord was collected by "milking" the umbilical cord after having separated it from the child. The estimations on specimens obtained in this way varied rather widely and therefore we tried to find out the factors which might be concerned in producing these variations. It was found that extracts prepared from the umbilical cord from which all blood had been removed, had no histaminolytic action whereas that of the amniotic fluid was very strong. Since the histaminolytic action of the plasma of blood collected from the umbilical cord was less than one hundredth of that of the amniotic fluid it is evident that even the slightest pollution of the sample with amniotic fluid may be of great consequence, particularly in view of the fact that the amount of plasma obtained from blood from the umbilical cord is comparatively small. Another factor which interfered early on in our investigations was the fact that haemolysis in blood collected from the umbilical cord occurs much more rapidly than in adults. The presence of haemolyzed blood corpuscles interfered with the assays of the histamine on the gut of the guinea-pig in almost all cases. If, before separating the umbilical cord from the

child and after carefully wiping the umbilical cord dry, the blood vessel was punctured under sterile conditions and the plasma immediately centrifuged and decanted, the values of the histaminolytic power showed far less variations than those which were obtained, when these precautions were not taken.

It would, of course, have been useful if we could have made estimations also on blood from the umbilical arteries. Owing to the rapid occlusion of the arteries after parturition, however, the quantity of blood collected was mostly so small that it did not permit of any accurate analysis. (After dissection of the umbilical cord it is, as a rule, not possible to obtain a sufficiently large amount of blood from the umbilical arteries by "milking".) Apart from the examination of blood from the umbilical arteries we studied in some cases also blood from the foetal heart and liver. (These organs were obtained on the occasion of a legally induced abortion at the sixth month of pregnancy.)

As a rule, the amniotic fluid was collected by puncturing the amniotic sac. This procedure eliminates the danger of impurities. It was found that the admixture of blood, even in very small quantities, interfered with the assays, probably through haemolysis of the blood corpuscles. We investigated also the histaminolytic power of the maternal urine (WERLE, 1940) which may readily cause impurity of the amniotic fluid collected in the vulva. It was found that the maternal urine contained matters which made its assay on the gut of the guinea-pig impossible. In most cases the amniotic sac was punctured on the occasion of a cesarean section which, at an early stage of pregnancy was performed for legally induced abortion. Since it has been demonstrated that bacteria may interfere with the estimation of the histaminolytic power if the histamine inactivation is very weak (WERLE, 1940, AHLMARK, 1944) — the incubation time is then 24 hours at a temperature of 37° C — we tested also some samples for bacteria. These tests showed that both the blood from the umbilical cord and the amniotic fluid collected in the manner described above were free from bacteria.

Early on in our investigations, the histaminolytic action of the entire placenta was studied. After having removed as much superficial blood as possible, separated the umbilical cord and the membranes, the placenta was ground, extracted with physiological saline solution and the histaminolytic power of the extracts estimated. Since this procedure takes rather a long time the placenta was cut into small pieces which were extracted and studied. ANREP, BARSOUM and IBRAHIM (1947) used a similar technique and emphasized that the extracts should be prepared as soon after delivery of the placenta as possible because they have found that keeping this organ over night, even at a low temperature, diminishes its histaminolytic action. I have investigated the histaminolytic action of several pieces of one and the same placenta after having kept them in the ice-chest for different lengths of time but have not detected any decrease in their histaminolytic action during the first 48 hours with the exception of placentas which, in spite of having been kept at a low

temperature, began to smell badly (this happened, for instance, in placentas delivered after the intrauterine death of the foetus). In order to avoid the effect which a prolonged keeping of the placenta might have on its histaminolytic power I prepared the placental extract as soon as possible after the delivery of the placenta.

It is well-known that the placenta consists of a foetal and a maternal portion. The maternal portion represents the decidua compacta which lines that part of the placenta which has been attached to the uterine mucosa. In the present study these two portions were separated and the histaminolytic power of each portion was estimated separately. It was found that there was a remarkable difference between their histaminolytic action, the histaminolytic power of the decidua compacta being far stronger than that of the foetal portion. This explains why the examinations of different pieces of placental tissue yield grossly varying values which depend on the quantitative relation between the decidua compacta and the foetal placental tissue present in a given piece. Owing to the fact that the decidua compacta is a thin and brittle membrane its dissection from the foetal portion presents great technical difficulties. It is almost impossible to separate it from the foetal portion of the placenta without inadvertently also removing some of the latter tissue. Besides, the decidua penetrates into the placental tissue in the form of septa. I have tested several methods but so far the purely mechanical method of dissecting the decidua compacta into small pieces with the help of small pincers, a knife and a pair of scissors has in our hands yielded the best results. The more skillful we became in applying it the greater was the difference between the histaminolytic actions of these two tissues.

It should be mentioned that the possibility was taken into account that the difference observed between the histaminolytic actions of the decidua compacta and the foetal portion of the placenta might be only a seeming one owing to the difference between their structures, sap content, and so forth. Large vessels and fibres of connective tissue were therefore as far as possible removed from the placental tissue before weighing it. No significant difference in the dry weights of these two tissues was observed. For the purpose of furnishing further evidence that the difference between the histaminolytic actions of the decidua compacta and the foetal portion of the placenta was genuine and not merely a seeming one, possibly due to a difference in the specific weights of these tissues, we examined pieces of placenta lined with the decidua compacta and also pieces from which this tissue had been removed. Any bearing which the thin decidual membrane might have on the specific weights of the individual pieces is here negligible. It was found that the histaminolytic power of all pieces lined with decidua compacta was much stronger. The study of a limited number of pregnant women whose pregnancies were interrupted at an early stage of gestation showed that the histaminolytic power of the decidua was not merely due to diffusion. When clearing out the uterus by means of cesarean incision in these cases the ovum (the foetus and the placenta) and the decidua

vera could each be removed separately. The estimations of the histaminolytic action showed that the decidua vera had a stronger histaminolytic action than the placenta which, in these cases, consisted of both chorionic villi and decidua basalis. In placentas delivered at an early stage of pregnancy it was not possible macroscopically to differentiate between these two tissues.

Results.

I. Blood from the Umbilical Cord.

Table I.

The Histaminolytic Action of Blood Collected from the Umbilical Cord of a Full-term Child.

Case	Blood from the umbilical cord γ/ml./hour (Ven. umbilical.)
G. A.	< 0.006
M. B.	< 0.006
M. H.	0.012
K. A.	< 0.006
S. J.	< 0.006
S. H.	0.012
E. B.	< 0.006
I. L. S.	< 0.006
E. L.	0.018
A. H.	0.012

Tables I and II show that with the exception of one case where the findings suggested that the histaminolytic action of the blood collected from the vena umbilicalis was $\leqq 0.024$ γ/ml./hour, the values ranged between < 0.006 and 0.024 γ/ml./hour. The histaminolytic action of the maternal blood ranged between 2.52 and 9.6 γ/ml./hour. (This high value was found in the mother of twins.) Hence, in one case the histaminolytic action of the blood collected from the umbilical cord was approximately one hundredth, in one case, two hundredths and in the remaining cases, less than three hundredths of the maternal blood. There does not seem to exist any correlation between the magnitude of the histaminolytic power of the maternal blood and that of the foetal blood.

In two cases we investigated also the histaminolytic action of blood from the umbilical arteries. It was found to equal 0.012 γ/ml./hour. In addition, blood from the foetal heart and liver was investigated in three cases. The histaminolytic power measured < 0.006 γ/ml./hour.

Table II.

The Histaminolytic Action of Blood from the Umbilical Cord of a Full-term Child as compared to that of the Maternal Blood.

Case	Blood from the umbilical cord γ/ml./hour		Maternal blood γ/ml./hour
	Ven. umbil.	Art. umbil.	
D. Å.	< 0.006		2.58
H. S.	< 0.006		> 6.9
R. S.	0.024		9
R. E.	0.006		8.62
I. L.	< 0.006		2.7
M. G.	0.012		7.56
E. J.	< 0.006		3
A. J.	< 0.006		6.78
B. J.	0.012		4.26
M. S.	< 0.006		2.58
R. P.	0.012		2.52
E. V.	≧ 0.024		4.08
A. K.	0.012		9.6
Twins	0.012		
B. N.	0.012		7.08
S. K.	< 0.006		2.76
K. P.	< 0.006		9.12
R. T.	< 0.006		7.62
A. J.	0.012		8.7
M. H.	< 0.006	0.012	2.88
A. L. H.	0.018	0.012	

In the group of healthy men and non-pregnant women the histaminolytic action of the blood was ≦ 0.006 γ/ml./hour. This value agrees with that determined by AHLMARK (1947).

Hence, the histaminolytic power of blood collected from a full-term child seems to be either approximately on a level with that collected from healthy men and non-pregnant women or somewhat higher. These observations do not agree with those made by other authors (see above) who reported that the histaminolytic action of blood collected from the umbilical cord was 30 % of that of the maternal blood.

II. Amniotic Fluid.

The histaminolytic action of the amniotic fluid was estimated in 23 cases: in most of them we determined the histaminolytic power also of the maternal blood. The specimens of amniotic fluid were collected either on the occasion of a legally induced abortion performed on women at the third to sixth month of pregnancy or on the occasion of parturition at the tenth month

of pregnancy.[1] In two cases no histaminolytic action of the amniotic fluid was detected. In the remaining cases the histaminolytic action of the amniotic fluid was on a level with the maternal blood. Graphically represented, the curve corresponded to that published by AHLMARK. In the group of patients at an early stage of pregnancy the values of the histaminolytic action of the amniotic fluid varied, *i. e.*, in some cases they were higher and in some lower than those of the histaminolytic action of the maternal blood. In the group of cases where maternal blood and amniotic fluid were collceted on the occasion of parturition the histaminolytic action of the amniotic fluid was always somewhat weaker than that of the maternal blood. In this group which included six cases, the mean values were: maternal blood = 3.6 γ/ml./hour, amniotic fluid = 3.06 γ/ml./hour.

In the two cases where no histaminolytic action of the amniotic fluid was detected, the amniotic fluid was in one case collected in the vulva. The possibility, therefore, cannot be excluded that some impurity of the specimen interfered with the test. In the other case the amniotic fluid was collected from a woman who was in the beginning of her fourth month of pregnancy (length of the foetus = 10 cm.). The record of this case states: "The colour of the amniotic fluid is unusually yellow." At the present stage of our knowledge it is difficult to determine whether this unusual colour of the amniotic fluid should be interpreted as a sign that some matters were present which interfered with the assay of the sample. In contrast to the matters present in the maternal urine the urinary matters which the amniotic fluid contains, have hitherto not interfered with its assay.

The observations presented in this paper do not agree with those made in other quarters that the amniotic fluid has no histaminolytic action.

III. Placenta.

As mentioned above, the values of the histaminolytic action of the placenta are not quite reliable. They show, however, that it increases markedly during the first half of pregnancy and that it does not increase further in connection with parturition. (In 5 cases we estimated also the histaminolytic action of the maternal

[1] The length of pregnancy is considered to embrace ten months, one month corresponding to four weeks.

Table III.

The Relation between the Histaminolytic Action of the Placenta and Length of Pregnancy (and the Maternal Blood).

Case	Month of pregnancy	Length of foetus	Placenta (foetal portion + maternal portion) γ/g./hour	Maternal blood γ/ml./hour
G. T.	II		1	
I. F.	II		14	
A. L.	II—III		6	0.05
M. M.	III	7 cm.	8	0.3
M. F.	IV	12 cm.	4	1.6
R. E.	V	18 cm.	52	
K. B.	V	20 cm.	127	
H. M.	V	22 cm.	43	
E. R.	V	23 cm.	103	
V. E.	V	24 cm.	73	2.8
A. E.	V	24 cm.	127	2.2
M. E.	V	25 cm.	55	
B. V.	X		92	
G. H.	X		84	

blood. For purposes of comparison we included the values obtained in these cases in Table III.)

Table IV shows that there is a marked difference between the histaminolytic actions of these two tissues. In one case the ratio between the magnitude of the histaminolytic action of the maternal portion and that of the foetal portion is 121: 1.

In some cases the values of the histaminolytic action of the foetal portion of the placenta are on a level with those of the maternal blood. The great variations which the figures show are probably due to technical difficulties. The powerful action of the decidua compacta (of all tissues hitherto examined it has the strongest histaminolytic action) suggests that this tissue is the source of the histaminolytic enzyme in pregnant women.

The histological picture of the decidua compacta also suggests that a significant internal secretion takes place in this tissue. The cells of the decidua compacta bear a close resemblance to those present in endocrinous glands (FUJIMURA, 1921, BROMAN, 1927).

Assuming that the decidua compacta is the source of the histaminolytic enzyme, the idea suggests itself that a depression or cessation of the function of the foetal portion of the placenta is not immediately followed by a decrease in the production of the histaminolytic enzyme. In order to investigate whether this assumption holds good, we estimated the histaminolytic action

Table IV.

The Relation between the Histaminolytic Action of the Maternal Portion of the Placenta, i. e., Decidua Compacta, and that of the Foetal Portion of the Placenta.

Case	Month of pregnancy	Maternal portion (Decidua compacta) γ/g./hour	Foetal portion γ/g./hour
A. K.	IV	177	19
A. B.	IV	295	11
V. I.	V	414	18
E. S.	V	336	4
E. T.	V	168	6
E. M.	V	1,182	83
B. A.	V	484	69
R. E.	V	414	22
B. O.	VI	163	22
K. J.	VII	618	31
R. L.	IX	167	32
V. B.	X	188	44
C. V.	X	226	36
V. L.	X	141	14
I. L. S.	X	446	32
B. S.	X	78	13
I. J.	X	428	98
B. L.	X	492	47
A. R.	X	45	11
M. A.	X	486	9
Twins	X	256	7
B. S.	X	148	34
E. S.	X	428	11
A. L. K.	X	231	4
S. M.	X	181	11
M. A.	X	292	8
Twins	X	251	7
K. H.	X	141	19
G. P.	X	247	15
M. L.	X	1,104	11
T. F.	X	484	4
R. S.	X	1,053	23
I. E.	X	364	12
K. L.	X	948	20
E. G.	X	98	4
I. E.	X	273	33
E. W.	X	432	8
B. S.	X	331	10
U. B.	X	410	46
M. B.	X	116	7

of the maternal blood in a group of cases where the foetus had died in the uterus. It was found that simultaneously with the discontinuance of the circulation the function of the villi ceases and regressive changes take place in the foetal portion of the placenta. A shift of the proportion of the functional power of the

decidua to that of the villi to the advantage of the decidua occurs (HINSELMANN, 1925). The regressive changes affect the decidua later and only gradually. In order to determine the exact time of the death of the foetus in the uterus a group of women at a late stage of pregnancy were examined.

This group comprises 15 cases in which intrauterine death of the foetus had occurred at between the 7th and 10th months of pregnancy. With the exception of one case (No. 1), the death of the foetus was verified by the cessation of the foetal heart sounds and by the fact that the foetus was macerated at delivery. In case 1 the foetal heart sounds ceased nine and a half hours before parturition. At delivery the foetus was dead but not macerated. Since it is not within the scope of this paper to discuss these cases in greater detail the principal features of their histories are presented in tabular form. Table V.

A histaminolytic action of the maternal blood after the death of the foetus was detectable in cases 1—5 the values being 3.24, 4.32, 5.76, 3.96, 3.9 γ/ml./hour, respectively. These values agree with those published in other quarters (see above) and also with the values which AHLMARK considered normal.

In 3 cases (cases 6—8) shown in Table V, the histaminolytic action of the maternal blood was estimated on two occasions during the interval between the death of the foetus and parturition. In a group of cases where pregnancy was associated with a disease (*e. g.* nephropathy grav. diabetes mellitus) which may threaten the life of the foetus in the uterus, it was possible in 7 cases (cases 9—15) where the foetus died, to establish the values of the histaminolytic action of the maternal blood before the death of the foetus and in 6 of them both before after the death of the foetus.

The observations on these 15 cases do not agree with those reported by other authors who observed that intrauterine death of the foetus at a late stage of pregnancy was accompanied by a marked depression of the histaminolytic action of the maternal blood. The findings in this study suggest rather that it increases in connection with the death of the foetus. According to AHLMARK the histaminolytic action of the maternal blood increases before parturition under normal conditions. In most of the cases here presented this increase was more marked than that AHLMARK observed.

In a case of hydatid mole I found that the cystic mass had no

Table V.

Principle Features of the Histories of 15 Cases where the Foetus died at between the 7th and 10th Months of Pregnancy.

Case	Last menstruation	Date 1947 Histaminolytic action γ/ml./hour	Date 1947 Death of foetus	Date 1947 Histaminolytic action γ/ml./hour	Date 1947 Parturition	Weight of foetus, g.	Length of foetus, cm.	Cause of the death of the foetus.
1) R.A.	1/12 46		31/7	31/7 3.24	31/7	1,380	42	Eclampsism
2) G.A.	20/10 46		20/6	1/7 4.32	1/7	2,300	48	Unknown
3) A.J.	22/3 47		7/10	24/10 5.76	29/10	1,800	41	Nephropathy
4) M.O.	13/4 47		(about) 1/12	12/12 3.96	15/12	1,500	44	Unknown
5) I.L.	12/3 47		(about) 13/12	15/12 3.9	15/12	3,070	52	Unknown
6) I.B.	22/9 46		(about) 6/4	8/4 6.66 10/4 7.26	13/4	—	34	Unknown
7) V.D.	4/12 46		31/7	1/8 2.58 4/8 3.06	18/8	1,025	39	Unknown
8) A.S.	22/3 47		12/11	18/11 4.02 24/11 4.44	25/11	3,150	49	Erythroblastosis
9) A.T.	6/12 46	25/8 4.32	30/8	1/9 2.88 4/9 3	5/9	2,300	42	Nephropathy
10) L.A.	31/12 46	5/9 6.54 22/9 9.36 / 12/9 6 29/9 9.24 / 18/9 8.34	6/10	6/10 9.78 7/10 11.88	8/10	2,950	50	Nephropathy
11) M.P.	15/3 47	5/9 2.1 / 13/9 2.1 / 24/9 2.4	25/9		25/9	938	38	Nephropathy
12) M.H.	5/2 47	11/9 1.08 / 18/9 1.26	21/9	22/9 0.84 23/9 1.24 24/9 0.84 25/9 1.02	2/10	1,200	40	Eclampsia
13) B.L.	(about) 7/2 47	22/9 2.64 / 8/10 3.48	12/10	15/10 3.9	16/10	1,000	37	Nephropathy
14) B.L.	1/1 47	20/9 1.5	26/9	25/9 1.86	30/9	2,300	48	Diabetes mellitus
15) M.L.	28/2 47	10/10 1.26 / 12/10 1.68 / 12/11 2.64	12/11	14/11 2.82 15/11 1.74	16/11	1,500	40	Nephropathy

histaminolytic action whereas the histaminolytic action of the maternal blood equalled that of the maternal blood during a normal pregnancy. This is further evidence in support of the assumption that the foetal elements are not concerned in the production of the histaminolytic enzyme.

Discussion.

The observations reported in this paper suggest that the histaminolytic enzyme is mainly formed in the decidua. They contribute thus to a better understanding of the importance of the increase in the histaminolytic action during pregnancy to which the profession has hitherto given little attention.

The assumption that the decidua is the main source of the histaminolytic enzyme may furnish the explanation why the death of the foetus does not produce a decrease in the histaminolytic action of the maternal blood. It is the function of the foetal portion of the placenta which ceases first in these cases.

Admittedly, the number of cases studied in this paper where the foetus died in the uterus is too small to permit of definite conclusions. Nevertheless, the assumption seems to be warranted that neither a threatened viability nor the death of the foetus produce immediately a decrease in the histaminolytic action of the maternal blood.

The results of these investigations suggest that in connection with the intrauterine death of the foetus an increase in the histaminolytic action of the maternal blood takes place instead of a decrease. A rapid decrease of the histaminolytic action of the maternal blood does not occur until parturition when the decidua is removed or breaks down (AHLMARK and others). Any damage to the decidua causing an impairment of its function might cause a decrease in the histaminolytic action of the maternal blood. Investigations into these problems are in progress with special consideration taken to changes in the decidual function depending on disorders in the balance of hormones and vitamines.

Summary.

The histaminolytic action of blood from the umbilical cord of a full-term child is very weak; as a rule, it is less than one hundredth to one thousandth of the maternal blood. It is either on a level or somewhat higher than the histaminolytic action of blood from men or from non-pregnant women.

The histaminolytic action of the amniotic fluid seems to be comparatively strong. It follows largely the maternal blood curve published by AHLMARK. After a rapid increase during the first half of pregnancy it remains approximately at the same level until parturition.

The histaminolytic action of the placenta is very strong. It increases rapidly during the first half of pregnancy.

The maternal portion of the placenta, *i. e.* the decidua compacta, has a much stronger histaminolytic action than the foetal portion. Of all organs hitherto studied it has been found to have the strongest histaminolytic action.

The intrauterine death of the foetus during the second half of pregnancy does not immediately cause a decrease in the histaminolytic action of the maternal blood.

The observations suggest that the histaminolytic enzyme is formed in the decidua.

My thanks are due to Dr. AHLMARK for his great interest in my work. His constructive criticism and wide experience in this field of research have been an invaluable aid in carrying out my investigations.

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From the Physiological Department, Karolinska Institutet.

Liberation of Histamine and Sympathin by Stimulation of Isolated Splenic Nerves from Cattle.

By

U. S. v. EULER and A. ÅSTRÖM.

Received 10 April 1948.

CALABRO repoited in 1933 that when the cut end of a sympathetic nerve was immersed in Ringer's solution and stimulated, the fluid elicited a positive inotrope and chronotrope action on the isolated frog's heart. Shortly afterwards GADDUM and KHAYYAL (1935) observed that a substance with adrenaline-like properties was released into the surrounding Locke solution when isolated cat's nerves containing adrenergic fibres were stimulated electrically. Though GADDUM, KHAYYAL and RYDIN (1937) later expressed doubts as to the physiological significance of these results, analogous observations were reported by BABSKY in 1938 who found that electrical stimulation of the sciatic or sympathetic nerves in situ caused a substance with properties similar to adrenaline to pass from the cut end of the nerve into the fluid in which it was dipped.

In experiments with cat's nerves LISSÁK (1939) found that an adrenaline-like substance was liberated by stimulation of nerve trunks containing adrenergic fibres. Sympathetic ganglia under the same conditions released both acetylcholine and adrenaline.

Until recently it has been generally agreed that acetylcholine and adrenaline were the two pharmacologically active substances present in nerves and capable of producing effects on liberation from nerve endings.

Recent experiments have demonstrated, however, that adrenergic nerves of cattle contain a sympathomimetic compound which is indistinguishable from l-noradrenaline (EULER, 1946, 1948 a, b).

The latter compound has been termed Sympathin N (EULER, 1948 c) in order to differentiate it from adrenaline (Sympathin A) which may be liberated from certain adrenergic nerves (GADDUM and KWIATKOWSKI, 1939). It is most likely that the substance believed to be adrenaline in some previous reports has actually been l-noradrenaline since the methods used for characterization of the liberated substance would not have allowed of differentiation between the two compounds, except in the experiments of GADDUM and coworkers.

In 1943 KWIATKOWSKI showed that histamine appeared in nervous tissue in varying concentrations which actualized the idea that certain nerves contained histamine as a specific ergone (histaminergic nerves, UNGAR, 1935). His findings have been confirmed, and, in addition it has been shown that certain autonomic nerves in cattle may contain as much as 100 μg histamine dihydrochloride equivalents per g of fresh tissue (EULER 1948 c).

It seemed therefore of interest to study whether histamine, like the sympathins or acetylcholine in various nerves, was liberated during stimulation from the isolated trunk of a nerve containing histamine in large amounts, such as the splenic nerves of cattle.

Methods.

Splenic nerves from cattle were dissected out in pieces of 8—12 cm shortly after death and kept moist at room temperature. The following procedures were used to demonstrate the liberation of substances from the cut end of the nerves.

a) The whole nerve, except the portion on the electrodes, was submerged in 1—2 cc Ringer's solution.

b) The nerve was placed in a bent glass tube and the cut end, extruding about 5 mm from the tube, was allowed to dip into a test bath containing a piece of guinea-pig's intestine. After one or more washings the nerve was stimulated at the other end (5—10 cm distant from the end placed in the bath) and the effects recorded.

c) The freshly cut end of a nerve, kept moist and warm, was allowed to dip a few mm into a small vessel containing 1—3 ml Ringer's solution. After 2 minutes the nerve was transferred to another vessel with Ringer's solution and after another 2 minutes to a third vessel. During this last period the nerve was stimulated at the other end. The procedure was repeated, usually 5—10 times each time with a freshly cut surface. The fluids were then tested as before on isolated guinea-pig's intestine, or on cat's blood pressure.

Stimulation was effected in most cases with a class inductorium and the minimal effective strength was used. In other experiments square

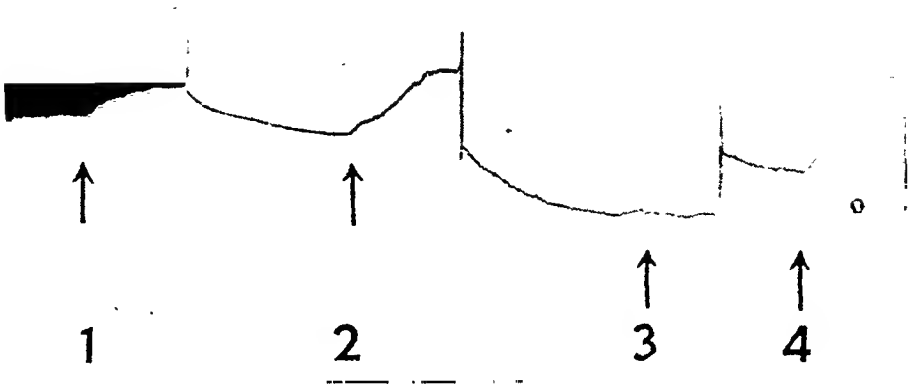

Fig. 1. Isolated guinea-pig's ileum. Tyrode. 1 and 3. Laking fluid (1 cc) from 10 cm splenic nerves of cattle immersed for 10 min. without stimulation. 2 and 4. Laking fluid (1 ccm) from the same nerve, immersed for 10 min., stimulated at one end (not immersed).

currents of about 10 msec. duration and a frequency of about 20/sec. were used.

Tests were made on the isolated guinea-pig's ileum in Tyrode's solution, containing $^1/_2$ the usual amount of $MgCl_2$, or in some experiments without $MgCl_2$. In order to exclude acetylcholine effects atropine sulphate was added to the bath to a concentration of 10^{-7}. The effects caused by the addition of fluids were tested before and after antergan which was added to a final concentration of 10^{-6}.

In some instances the blood pressure of the cat was used for testing, after a previous dose of 0.1 mg gynergen and 1 mg atropin per kg.

Results.

In the first series of experiments the test fluid was in contact with the whole nerve except for the part on the electrodes. After a primary period of 10 minutes without stimulation the nerve was stimulated for 10 minutes and the effects compared.

Figure 1 shows the stimulating effect of the fluid collected during the period of stimulation, whereas only a negligible effect is observed with the corresponding amount of fluid from the rest period.

In the majority of cases the effect of the fluid from the stimulation period elicited a stimulating action, but in some cases an inhibitory effect was observed and in others a compound effect. The inhibitory action is probably due to liberated sympathin N (noradrenaline) which is present in large amounts in these nerves.

In the second series of experiments the nerve was stimulated

Fig. 2. Isolated guinea-pig's ileum. Tyrode. 6 cm splenic nerve of cattle inserted in a glass tube, one end dipping in the bath and the other end stimulated for $1^1/_2$ and $2^1/_2$ minutes respectively. Washing at x.

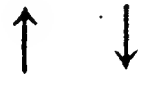

Fig. 3. Same arrangement as in fig. 2. Between arrows stimulation for $1^1/_2$ minute of one end of the nerve.

while the other end was submerged a few mm into the bath with the guinea-pig's ileum. Immediately upon placing the cut end of the nerve in the bath there occurred as a rule a contraction of the intestine, probably owing to diffusion of active substances into the bath from the nerve. After 1 or 2 washings, however, when the intestinal strip showed no activity, stimulation was begun. The result was even here, in most cases, a stimulation of the activity, partly appearing as waves with small amplitude and partly as an increase of the tone level (Fig. 2). In some cases an inhibition was observed (Fig. 3).

The method described under c in the technique was used in a number of experiments. Generally the fluid obtained during

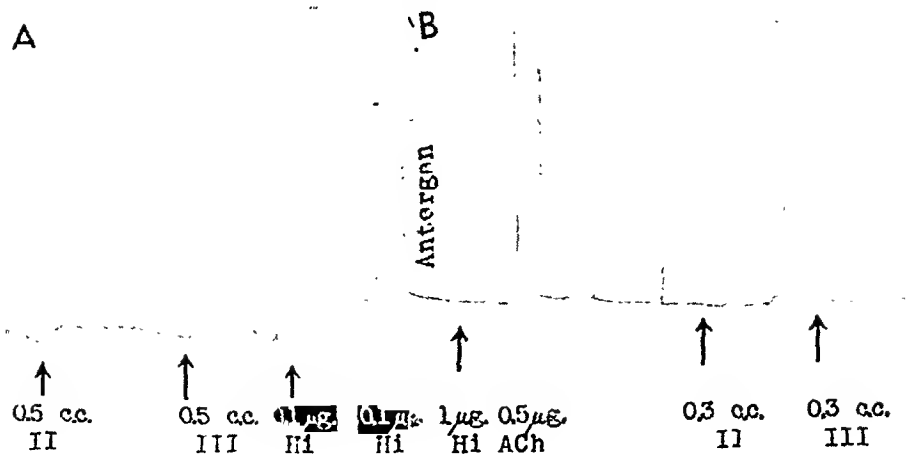

Fig. 4. Isolated guinea-pig's ileum. Tyrode. Distal tip of splenic nerves of cattle immersed in Ringer's solution for periods of 2 minutes each. II = fluid from second lakings without stimulation. III = fluid from third lakings with stimulation of central end. Atropine 10^{-7} in bath during, A, Antergan 10^{-6} in bath during B.

the first period of rest with the freshly cut nerve had a fairly strong stimulating action whereas the fluid from the second period was much less active, and was used as a control for comparison with the fluid from the following stimulation period (Fig. 4). The "stimulation fluid" caused an effect which was definitely greater than that seen with the fluid from the second period. Since care was taken to prevent fluid from the stimulated part to reach the vessel, it must be concluded that the stimulation had liberated some substance producing contraction of the guinea-pig's ileum.

In order to obtain further knowledge as to the nature of the liberated substance the fluid was tested anew after treatment of the intestine with antergan. This resulted in a complete annulment of the sensitivity to histamine in the doses used without interfering with the sensitivity to acetylcholine. On adding the test fluids no stimulating action whatever could be observed, which strongly indicates that the active substance is histamine (Fig. 4 B). On the other hand a slight inhibition could be observed, probably due to sympathin.

On the cat's blood pressure the effect of stimulation on the isolated nerve could also be demonstrated. Fig. 5 shows the effect of injection of the Ringer solution (1 cc) from the resting periods and the periods of stimulation. The effect is in accordance with the

action of histamine which supports the assumption that the liberated substance is histamine itself.

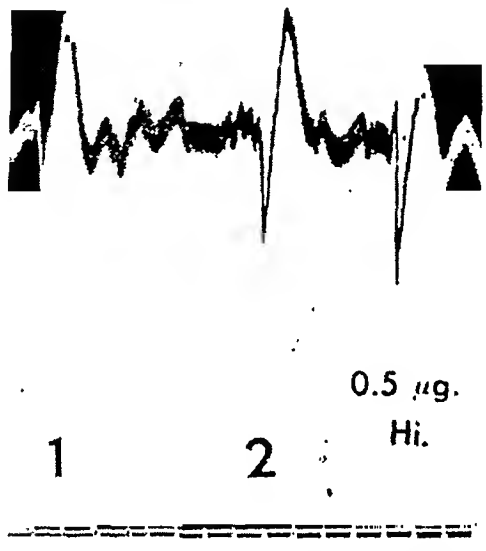

Fig. 5. Blood pressure, cat. Chloralose. Atropine. 1. Fluid (1 cc) from second lakings of end of splenic nerve of cattle. 2. Fluid (1 cc) from second lakings obtained in the same way but with stimulation of other end. Hi = histamine dihydrochloride. Time $^1/_2$ min.

Comment.

Obviously the assumption that certain remote effects observed after nerve stimulation in vivo are due to the liberation of substances from the nerve endings is strongly supported by the findings that these nerves actually contain substances producing effects of the same kind as those observed on stimulation (WITANOWSKI, 1925, CHANG and GADDUM, 1933, LISSÁK, 1939, EULER, 1946).

The evidence for liberation of histamine from the isolated nerve trunk of splenic nerves in the present work is based on the following data.

1. Stimulation liberates a substance producing contraction of the isolated guinea-pig's gut.

2. This action is annulled by antihistaminic substances in concentrations which do not impair acetylcholine action.

3. The liberated substance lowers the blood pressure of the cat after atropine.

The fact that extracts of fresh nerves contain histamine in amounts of 40—100 μg per g may be regarded as strong indirect evidence.

It appears therefore highly probable that the histaminelike substance liberated by stimulation of splenic nerves from cattle is histamine. The above findings also support the assumption that the histamine liberated by antidromic stimulation of sensory nerves in vivo (LEWIS and MARVIN, 1925, KWIATKOWSKI, 1943) is really derived from the nerves themselves.

A few points concerning the process of liberation of ergones from the splenic nerves merit special mention. It has been noted that only fresh nerves, kept not more than a few hours after the death of the animal, are capable of responding to stimulation by liberation of the ergones studied. Usually it was necessary to have a freshly cut surface in order to get a repeated response to stimulation when the end was dipped in the test bath containing the guinea-pig's intestine.

Since sympathin as well as histamine is present in the splenic nerves of cattle in considerable amounts, it might be expected that both substances were liberated. Our results indicate that such is the case, although certain differences were noted. Thus sympathin was usually detected only during the first stimulations of a quite fresh nerve whereas histamine as a rule could be found as a result of stimulation even after some hours.

Though undoubtedly sympathin as well as histamine is initially liberated by simple leakage from the cut end of the nerve and also released by disintegration of the tissue, as held by GADDUM, KHAYYAL and RYDIN (1937), we believe that in our experiments the liberation of the substances is effected by the stimulation. This is based on the following observations: (1) after a period of stimulation the amount of active substance in the washing fluid was larger than after the preceding second resting period, (2) after repeated changes of fluid in the intestinal bath, when the gut was quiescent, stimulation produced a definite effect with a short latency.

In many instances it was noted that stimulation of the nerve, the end of which was immersed in the intestinal bath, caused a gradual increase of tone of the isolated gut together with rhythmical movements (Fig. 2). Whether these are due to histamine or some other substance cannot be determined as yet but it will be recalled that KWIATKOWSKI (1943) observed effects of much the

same kind on perfusion of guinea-pig's intestine with fluid deriving from a cat's hind-limb after stimulation of the posterior roots.

The results reported in the present paper seem to warrant the conclusion that certain autonomic nerves, such as the splenic nerves of cattle, which consist predominantly of postganglionic sympathetic nerves having the typical electrical properties of C-fibres (LUNDBERG, 1947) contain true histaminergic fibres, capable of liberating this ergone. This presents new problems as to the partaking of histamine as chemical mediator of nerve activity in the activity pattern of autonomically innervated organs and other processes under normal and pathological conditions.

Summary.

The liberation of nerve ergones from electrically stimulated, isolated splenic nerves of cattle has been studied.

Stimulation of these nerves released one substance acting like sympathin N (l-noradrenaline) and another substance eliciting effects characteristic of histamine.

It is inferred that splenic nerves of cattle contain true histaminergic fibres besides noradrenergic fibres.

The significance of these findings for the activity of certain autonomically innervated organs is briefly suggested.

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From Universitetets Biokemiske Institut, Copenhagen.

On the Transformation of Histidine to Creatine by Animal Tissue in Vitro.

By

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Received 16 April 1948.

The present note is a sequel to one recently published (STEENSHOLT 1948), in which we investigated the effect of optical isomerism in the transformation of histidine and arginine. It was found that only the "natural" optical isomers, the l-forms, were converted into creatine by frog muscle tissue in vitro. In the present report we attempt to study some further details of the process by which histidine is transformed into creatine, and in particular we shall give some attention to the problem of finding inhibitors and activators for the enzyme or enzyme systems catalyzing the reaction in question, hoping thereby to gain some insight into the mechanism of the process.

Experimental Part.

The biological material was partly frog muscles and partly muscle tissue from rat, guinea pig and rabbit. The tissue was removed immediately after the death of the animal (which was brought about either by decapitation or by a blow on the head). The tissue was then finely divided and the resulting pulp was used in the experiments reported below.

The histidine and the methionine were both Hoffmann-La Roche products.

We first investigated whether also mammalian muscle is able to transform histidine into creatine, and did this by the same

procedure as the one used in our previous work with frog muscle. A typical experiment was accordingly carried out as follows: A glass vessel A contained 0.35 g rat muscle tissue (pulp) suspended in 6 ml phosphate buffer (pH = 7.0). Another vessel B likewise contained 0.35 g muscle tissue, 6 ml phosphate buffer and 25 mg l(—)-histidine (the mixture having the same hydrogen ion concentration as that in vessel A). Vessels C and D were prepared in the same way, but with l(—)-histidine replaced respectively by dl- and d(+)-histidine. The vessels were incubated at 37° C for 5 hours. At the end of this period were added 4 ml 10 per cent trichloroacetic acid and 2 ml n/1 HCl. After standing for 1 hour the reaction mixtures were centrifuged and 5 ml of the supernatant liquid were removed for determination of total creatinine by the method of BENEDICT-BEHRE-LANGLEY-EVANS-LEHNARTZ, which has also been applied in the previous work. Table 1 contains the results of three experiments on rat, guinea pig and rabbit muscle tissue. For brevity we give only the relative increase in total creatinine, expressed in per cent, in vessels B, C and D compared to vessel A.

Table 1.

Muscle tissue from	Relative increase in total creatinine in per cent		
	Vessel B	Vessel C	Vessel D
Rat	12.0	6.5	1.0
	14.0	7.5	0.0
	11.5	5.1	0.2
Guinea pig	10.2	5.4	0.0
	9.0	5.1	0.2
	14.1	6.4	0.0
Rabbit	14.2	7.0	0.1
	12.4	7.0	0.5
	13.5	6.2	0.2

The figures given in the last column are necessarily rather uncertain. The experiments were carried out on three different animals of each kind.

Similar experiments were also carried out at different hydrogen ion concentrations. Both for rat, guinea pig and rabbit muscle pH-activity curves were obtained which were closely similar to the curve previously obtained for frog muscle. In particular the optimum pH value is near 7.0. Numerical details are probably unnecessary and are therefore omitted.

We next investigated whether certain substances, which are known to act as methyl donators in some biological methylation processes both in vivo and in vitro, have any effect on the reaction under consideration here. Among such compounds we have, as is very well known, methionine, choline and betaine. To clear up this question the following experiment was carried out. A vessel A contained 0.4 g frog muscle tissue and 6 ml phosphate buffer (pH = 7.0). A vessel B contained 0.4 g muscle tissue, 40 mg l(—)-histidine and 6 ml phosphate buffer, the mixture having of course the same hydrogen ion concentration as that in vessel A. A third vessel C was prepared in identically the same way as B, but contained in addition 35 mg dl-methionine. The vessels were incubated at room temperature for three hours, and their contents then worked up and analysed as described above. The relative increase in total creatinine both in vessel B and in vessel C was 12.5 per cent; no difference between the reaction mixtures could be detected. The experiment was repeated with rat muscle tissue (with incubation at 37° C for four hours) with the same qualitative result. Replacement of methionine by choline and then by betaine proved equally ineffective in changing the yield of creatine. Finally the experiments were repeated for all three methyl donators and both kinds of muscle tissue at pH 6.0 and 7.8. At these hydrogen ion concentrations the increase in total creatinine was much smaller than at pH 7.0, as was of course to be expected in view of our previous results, but no evidence of any effect of the methyl donators on the formation of creatine could be obtained.

The rest of the present note will be concerned with the problem of inhibitors and activators for the transformation of histidine into creatine. We first investigated whether cyanide, borate and pyrophosphate had any effect on the process. This was done by essentially the same procedure as that used for studying the effect of methionine, choline and betaine, and will therefore not be reported in detail. The experiments were carried out in parallel both for frog and rat muscle tissue, with qualitatively the same results in both cases.

Potassium cyanide was added to the reaction mixtures in concentrations of 0.0001 m, 0.001 m, 0.005 m and 0.01 m. Sodium borate was used in the concentrations 0.001 m, 0.005 m and 0.01 m, and finally sodium pyrophosphate in the concentrations 0.01 and 0.05 m. The effect of these substances was examined at pH 6.2, 7.0 and 7.9, as were also the other inhibitors referred to be-

low. All three inhibitors proved ineffective; no difference was found between the contents of total creatinine in vessels B and C.

Sodium fluoride turned out to have an inhibiting effect. When used in concentrations of 0.01 m a 60 to 70 per cent reduction in the creatine formation was observed. Likewise the thiol compounds cysteine and glutathione were found to be inhibitors. Used in concentrations similar to that of sodium fluoride they produced reductions in the yield of creatine of the same order of magnitude.

Of heavy metal salts we investigated the effect of manganese chloride and nitrate, magnesium chloride, copper sulphate, ferrous and ferric chloride and nickel chloride. They were used in about the same concentrations as sodium fluoride, but no effect on the creatine formation could be detected.

Of the narcotics only veronal and urethane were investigated. They showed some inhibiting effect, when added to the reaction mixtures in amounts of about 3 to 5 mg.

Comments.

The results reported above indicate that the ability of transforming histidine into creatine is a fairly general property of muscle tissue. The experiments with methionine, choline and betaine show that these methyl donators have no effect on the yield of creatine. We are therefore probably justified in concluding, with some measure of certainty, that the reaction in question is not a methylation process in the usual sense of the word. As regards our negative results with cyanate, borate and pyrophosphate as inhibitors it may be mentioned that in a truly cyanide sensitive system concentrations as low as 0.001 m KCN should give a very considerable effect. This was not the case in our experiments. In particular, therefore, the process under consideration does not appear to be coupled with respiratory oxidation-reduction systems in the tissues, at any rate not with the cyanide sensitive ones. Likewise the heavy metals investigated above turned out to have no inhibiting effect. Whether some of them, for instance magnesium, have any activating effect, cannot be denied with certainty, since this metal may already be present in the tissues. Nickel is of course neither an inhibitor nor an activator. The effects observed with sodium fluoride and with the thiol compounds show that these substances are inhibitors

of the reaction under consideration. It would be tempting to interpret this as indicating that phosphatases and phosphorylations play some as yet unknown rôle in the intermediary processes leading from histidine to creatine, but this question must certainly await further study. The effect of the two narcotics does not seem to call for any special comment at the present time.

It is clear that many problems are left open for future investigation, and the writer hopes to return to some of them later. Some further experimental work is actually in progress and it is hoped to report on the results in the near future.

The writer is glad to express his best thanks to Professor Richard Ege for his generous hospitality.

Summary.

Muscle tissue from frog, rat, guinea pig and rabbit is found to transform histidine into creatine in vitro. The yield in creatine is uninfluenced by the presence of methionine, choline and betaine. Cyanide, borate and pyrophosphate do not affect the process. A few heavy metals are also studied and are likewise found to have no effect. Sodium fluoride, cysteine and glutathione are found to be inhibitors. Veronal and urethane have some inhibiting effect. The possible interpretations of these findings are briefly discussed.

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The Distribution of Glycogen in the Rabbit Liver.

By

BENT BARFOD.

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It is of cardinal interest to know whether or not glycogen is uniformly distributed in the liver. In several investigations the glycogen content of the whole liver has been estimated from a single sample. The interpretation of the results obtained depends on the validity of the assumption that glycogen is uniformly distributed in the liver.

Numerous workers have dealt with this problem, and many different species have been used for the experiments, but concordant results have not been obtained.

In 1925 NEUBAUER concludes that the glycogen percentage differs rather markedly from lobe to lobe, whereas MAGNUS-LEVY in the same year claims a uniform distribution.

CORI (1925) finds that the glycogen amount in the liver of the guinea-pig varies between 1 and 3 % from sample to sample. GREVENSTUK and LAQUEUR (1925) find a mean value of 0.26 % of glycogen in the right lobe of a rabbit liver against 0.21 % in the left lobe.

From the figures of BICHEL and NIGMANN (1929) it is seen that two samples from the same rabbit liver can differ as much as 29 %.

NIELSEN (1931) takes it for granted that the distribution of glycogen in the normal liver is uniform, but based on thorough investigations SCHEIFF (1931) concludes that the distribution of glycogen in liver from dogs, rabbits and tortoises varies greatly.

The most recent investigation by GOMORI and GOLDNER (1947) concludes that the distribution is not uniform.

Obviously the discrepancies are to a great extent due to differences in procedure. Although the necessity for the consideration of the post mortem glycogenolysis has been proved from investigations by TAYLOR (1908—09) and by MACLEOD and PEARCE (1910—11), none of the authors seem to have paid enough attention to the rapid onset of the glycogenolysis.

In all the mentioned investigations the liver samples are treated in various ways (divided, weighed etc.) before glycogenolysis is arrested, and the time that passes until this is done is never noted.

Thus the problem still seems to be unsolved.

In the present paper I have examined the possibility of eliminating various sources of error so as to make possible a definite conclusion whether or not glycogen is uniformly distributed in the liver.

Present Investigations.

The determination of glycogen is carried out according to GOOD, KRAMER and SOMOGYI (1933) with a few modifications.

Reagents:
Potassium hydroxide 30 %.
Alcohol 96 %.
Alcohol 55 % by volume.
Hydrochloric acid N 0.6.
Sodium thiosulphate N 0.002.
Potassium ferricyanide reagent:
Potassium ferricyanide analytical grade g 0.8245
Sodium carbonate cryst. analytical grade g 14.3
Water to 500 ml.
Iodide-zinc solution:
Zinc sulphate analytical grade 5 %
Sodium chloride analytical grade 25 %
For each series of analyses is added:
Potassium iodide analytical grade 2.5 %
to the quantity of the zinc-sodium chloride solution to be used.
Acetic acid analytical grade 3 %.
Solution of starch.

Procedure:
2 ml of 30 % potassium hydroxide solution are measured into 15 ml rubber stoppered centrifuge tubes provided with a copper wire for suspending them on the analytical balance on which they are weighed.

Liver samples of about 1 g are immersed in the potassium hydroxide solution, the tubes are stoppered and again weighed.

The stoppers are removed and the tubes are immersed in water bath which is brought to the boil.[1]

When the tissue has dissolved (1/2—1 hour) the tubes are removed from the bath and cooled. The glycogen is precipitated by adding 1.1 volume of 96 % alcohol. The tube is stoppered with a finger and inverted several times. Glycogen that may stick to the finger is scraped off carefully on the rim of the tube. It is then advantageous, as directed by CHRISTENSEN (1944) to add further 3—4 ml of 55 % alcohol, which renders the centrifugate quite clear. The tube is inverted as before. The tubes are heated cautiously in a water bath until the mixture begins to boil (about 82°, possible bumping) and are then cooled. This heating causes coarser flocculation of the precipitate and allows it to be centrifuged after 1 hour's standing. This may, however, be postponed for 24 hours without affecting the result.

The centrifuging is carried out at 3 000 rev. per minute for 10 minutes. The mother liquor is decanted and the remaining alcohol allowed to drain on a filter paper until the glycogen precipitate has become dry and transparent, indicating complete removal of the alcohol. This drying takes about 48 hours. Alcohol, if present, may disturb the reduction procedure later on. Heating of the centrifuge tube for a few minutes in a water bath, as directed by GOOD, KRAMER and SOMOGYI (1933) among others, does not remove all the alcohol with certainty.

5 ml of N 0.6 hydrochloric acid are then added to each tube, which is closed with a cone-shaped glass stopper (made from a test tube) to prevent evaporation. The tubes are placed in a water bath which is brought to the boil. The boiling is continued for 2 1/2 hours. After cooling the glass stoppers are removed and washed with water, which is added to the hydrolysates, and the mixtures are filtered through previously washed cotton wool. The filtrates are transferred quantitatively to measuring flasks, the volumes of which are governed by the quantity of the glycogen precipitate (25—500 ml). In less diluted hydrolysates the hydrochloric acid must be neutralized by addition of an equivalent amount of sodium hydroxide solution. The volume is made up with water and mixed and the still somewhat turbid liquids are filtered through the same cotton wool. The first part of the filtrate is discarded and the rest is kept for the reduction analysis.

The filtrates may be stored in the refrigerator in glass covered vessels to the following day without affecting the results.

After a preliminary titration in order to find out how large a quantity of the filtrate is to be taken for the analysis — the requirement of sodium thiosulphate solution must not exceed 1 ml — a suitable amount is transferred to wide test tubes (26 by 94 mm) and water is added to a total amount of 8 ml, if necessary.

2 ml of the potassium ferricyanide reagent are added and the mixtures are heated in boiling water bath for 15 minutes. Just before

[1] Pyrex centrifuge tubes were not obtainable; otherwise it would be preferable to place the tubes at once in a boiling water bath.

the titration 3 ml of the iodide-zinc solution and 2 ml of the 3 % acetic acid solution are added, and the mixture is titrated with N 0.002 sodium thiosulphate solution from a 5 ml micro burette using starch solution as indicator.

A blank determination is performed on all reagents, and the sodium thiosulphate solution is standardized for each series of analyses.

FUJITA and IWATAKE (1931) have stated a formula for the glucose determination by the ferricyanide method. This formula gives more accurate results than does the calculation method of HAGEDORN aud NORMAN JENSEN (1918, 1923). This has been confirmed in experiments in which I recovered on an average 99.5 % of an added amount of glucose using the formula of FUJITA and IWATAKE, but 106.4 % according to the HAGEDORN and NORMAN JENSEN method.

The amount of glycogen in the liver, determined as glucose, is therefore calculated according to the formula:

$$0.00696 \frac{F \cdot D \cdot (X-x)}{A \cdot B} \%$$

A: the weight of the liver sample in g.
B: the quantity of the filtrate used for the glucose determination in ml.
D: the volume to which the glycogen hydrolysate from the liver sample is diluted in ml.
F: the factor for the N 0.002 sodium thiosulphate solution.
X: milliliters of N 0.002 sodium thiosulphate solution required to titrate the blank.
x: milliliters of N 0.002 sodium thiosulphate solution required to titrate the analysis.

The presence of non-fermentable reducing substances in the glycogen hydrolysate is examined by measuring the decrease in reducing substances caused by yeast fermentation. The amounts of these compounds were found to be rather insignificant (about 5 % of the total reduction) and very consistent in various samples. No attention is therefore paid to this fraction in the present work.

Influence of Glycogenolysis.

The following experiment (I) indicates how soon it is possible to detect glycogenolysis that will affect the results.

A non-fasting rabbit is killed by a blow on the neck. The liver is removed and divided into 8 sections which are freed from blood by a piece of filter paper. The sections are divided into samples of about 1 g, which are placed in ice-cold potassium hydroxide solution. 25 minutes elapses between the time that the first and the last sample is placed in the potassium hydroxide solution, that is to say the average interval between each of the sections amounts to about 3—4 minutes.

The results are shown in Table 1.

Table 1.

The liver sections	Weight in g of the sections	Numbers of separate analyses	Mean value of separate analyses
I	12.138	10	9.18 %
II	5.988	5[1]	8.62 %
III	3.566	2[2]	8.51 %
IV	11.395	7	8.59 %
V	8.480	6	8.53 %
VI	9.858	6	8.39 %
VII	10.525	8	8.12 %
VIII	8.625	8	8.10 %

[1] 6 analyses spoiled.
[2] 7 analyses spoiled.

It is seen, that the difference between the values for the sections is largest at the beginning (0.56 %) and decreases later on (0.02 %).

The inconsistency of the results may be due to glycogenolysis.

Hence I carried out an experiment (II) in which the enzymatic processes during the preparation of the liver samples were arrested:

A non-fasting rabbit is killed by a blow on the neck, the liver is removed as quickly as possible, some incisions are made into the thickest parts, the organ is freed from blood and thrown into a Dewar flask containing liquid air. The whole procedure — from the killing of the rabbit until the liver is placed in the liquid air — takes less than 2 minutes. The liver is cut into pieces in the liquid air by a bone forceps. The samples of about 1 g are transferred to the previously weighed centrifuge tubes containing 2 ml of potassium hydroxide solution.

The whole liver (87.02 g) is divided into 66 samples. The frozen samples placed in the potassium hydroxide solution are weighed in 8 series. Due to the cold contents the tubes will become misted. The weighing therefore is carried out very quickly after careful removal of the moisture. Experiments have proved that the weight of a liver sample is the same in normal and in frozen state.

When a series of samples has been weighed, the tubes are immersed in a water bath which is brought to the boil. The liver not yet treated is kept in liquid air in order to prevent glycogenolysis.

The results are shown in Table 2.

Table 2.

Series A	Series B	Series C	Series D	Series E	Series G
12.28 %	12.53 %	12.26 %	12.18 %	12.51 %	12.23 %
12.62 %	12.21 %	11.77 %	12.24 %	12.10 %	12.30 %
12.62 %	12.72 %	12.60 %	11.69 %	12.20 %	12.33 %
12.24 %	12.39 %	12.30 %	12.30 %	12.51 %	12.08 %
12.46 %	12.33 %	12.31 %	12.45 %	12.00 %	12.16 %
12.09 %	12.29 %	12.45 %	12.00 %	12.25 %	12.23 %
12.43 %	12.25 %	12.29 %	12.30 %	12.09 %	
12.14 %	12.32 %	12.54 %	12.24 %		Series H
12.13 %	12.81 %	12.47 %	11.99 %	Series F	
12.35 %	11.87 %	12.39 %	12.12 %		11.69 %
12.39 %	11.91 %	12.34 %	12.22 %	12.15 %	12.52 %
12.67 %	12.03 %				12.32 %
					12.36 %
					12.27 %
					12.20 %

The mean value for all 66 single determinations is 12.27 g of glycogen per 100 g of liver. The standard deviation of the single analysis is $\pm$ 0.23 g which corresponds to $\pm$ 1.87 % of the result.

A similar experiment (III) in which the liver samples in the frozen state were crushed to a fine powder before transferring them into the potassium hydroxide solution, gave the following result:

Table 3.

9.94 %	10.10 %	10.00 %
9.87 %	10.22 %	10.24 %
10.00 %	9.98 %	10.25 %
9.87 %	9.92 %	10.33 %
9.89 %	9.91 %	10.06 %
10.06 %	10.00 %	9.82 %
10.22 %	10.28 %	9.86 %
10.09 %	10.15 %	10.03 %

In this experiment the whole liver is not analyzed but only 24 samples. The mean value for these is 10.05 g of glycogen per 100 g of liver. The standard deviation of the single determination is $\pm$ 0.15 g corresponding to $\pm$ 1.49 % of the result.

Hence it must be concluded that the glycogen distribution in the liver is uniform.

If there is any deviation, it must be within the error of the analyses.

Summary.

The sources of error in previous methods for determining liver glycogen are discussed.

The present analytical procedure is described in detail.

The influence of the glycogenolysis is demonstrated experimentally. It has been proved that the enzymatic processes must be arrested at once. Liquid air has been used for this purpose.

Based on experiments it is concluded that the distribution of glycogen in the liver of the rabbit is uniform.

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A Simplified Method for Measurement of Radioactive Corpuscle Samples for Blood Volume Determination.

By

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The measurement of the blood volume of man by labelling the corpuscles with ^{32}P was suggested first by HAHN and HEVESY (1940) and was described later in detail by HEVESY and coworkers (1944). The principle of this method is to draw blood from a patient (about 20 ml) and after adding c. 2 μ Curie of ^{32}P, shake it, for 2 hours at 37° C. About 15 ml of the labelled blood is then reinjected into the subject. When knowing the amount of injected corpuscles and the ratio of the activities of 1 g corpuscles injected (standard corpuscles) and 1 g sample corpuscles taken 5—10 min. after the injection, it is easy to calculate the amount of corpuscles present in the body.

It has lately become rather easy to obtain the radioactive phosphate necessary for these determinations and it seems to be of some interest to make the measurement of the samples more convenient. HEVESY et al. dissolved the corpuscles by boiling in nitric acid and precipitated the phosphorus after adding some carrier P as magnesium ammonium phosphate. The precipitate was transferred to perforated aluminium dishes by filtering, and was dried before measurement. The standard samples were treated in the same way, but only 1/100 g was precipitated. A simplification of the method was made by NYLIN (3) who only dried the corpuscles, powdered them in cellophane, and transferred a certain amount to aluminium dishes by weighing. For measuring

the standard samples he mixed a small amount of the dried sample with inactive dried corpuscles to get the right weight.

There still seems to be a possibility to improve the method and, therefore, we tried to measure the corpuscles without drying them. This can be done by using a dipping counter. However, we preferred to use a common bell-shaped counter with a thin mica window and to transfer the corpuscles to small cuvettes. The cuvettes were made of a stainless steel ring, 3.00 mm thick, with a conical hole in the side of the ring. The ends of the ring were covered with circular cover glasses, 0.1—0.2 mm thick (Fig. 1a). The glasses were sealed on to the ring by picein; the excess of seal was removed with toluene and, finally, the cuvette was heated with a weight pressing the glasses tight to the ring. The steel ring can be made with an accuracy of $\pm$ 0.02 mm, and the glasses for all comparable cuvettes must have a thickness not varying with more than a fraction of 1/100 mm. A change in glass thickness of 0.01 mm was measured to give a change in counting of 1.8 %.

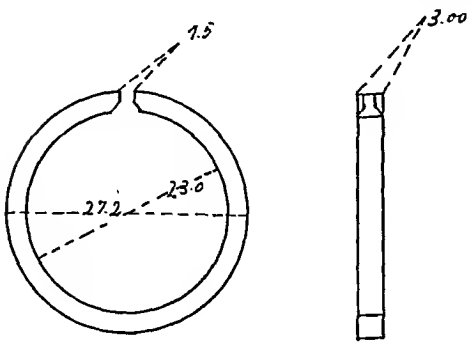

Fig. 1 a.

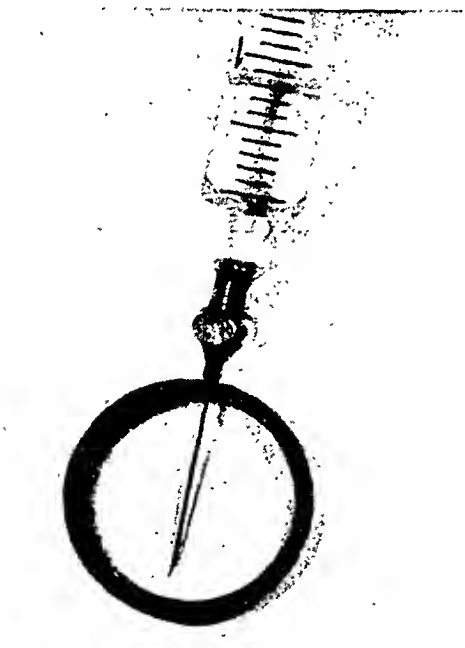

Fig. 1 b.

A cuvette is filled with corpuscles or solution through the small hole in the side by means of a medical syringe (Fig. 1 b) and it can be filled without getting air bubbles into the solution. If it is difficult to avoid air bubbles when filling the cuvette, the inside of the hole may be covered with a film of grease, using a

match moistened with vaseline. After filling, the hole can be closed with a small piece of scotch tape, if long measurements are necessary; for shorter measurements, the hole can be kept open. After use, the cuvettes with corpuscles can be cleaned with water by suction through a hypodermic needle; only when the activity has been very strong, the use of nitric acid may be necessary for cleaning.

The determination of the corpuscle volume is based on the comparison of the activities of a known volume of injected labelled blood corpuscles (standard corpuscles) and of blood corpuscles secured from the patient. If the activity of the injected blood corpuscles is too high for direct measurement, it is necessary to dilute the standard corpuscles. The measurement of standard samples can be performed by diluting 1 ml of standard corpuscles to 100 ml with distilled water. The dilution will hemolyze the corpuscles and from the mixture the cuvettes can be filled. The activity of the stroma is negligible (less than 0.1 %), but a correction should be made for the difference in the density of water and corpuscles (1.08). This correction was found to be 5 %; thus, the correct value for the standard corpuscles can be obtained by multiplying with 95 instead of 100. The correction can, however, be avoided by diluting with a glycerine mixture of the density 1.08 and adding a minute amount of saponine. Both ways have been tried and proved to work satisfactorily (glass thickness 0.16 mm).

When measuring solutions with minute amounts of P it is advisable to add some carrier P to the solution; otherwise, some error due to adsorption on the glass might occur. Another source of error which should be avoided is the growth of microorganisms in the solutions; this is important when working on solutions poor in P. The K in the glass of the cuvettes causes a small rise in the background, but this fact can be allowed for by using a cuvette filled with distilled water when measuring the background.

The advantage of using cuvettes instead of a dipping counter is that the samples can easily be measured by automatical changing. Furthermore, there is no risk of contamination of the counter. Even if the volume of the cuvettes varies by 2—3 % the accuracy of the measurements is better than 1 % due to the self-absorption in the sample. The K content of the corpuscles is too small as to cause difficulties if samples stronger than 50 counts/min. are measured.

This technique of measurement can be used for solutions of radioactive substances, when the rays have sufficient energy. It is, however, unsuited for measurement of weak rays. The sensitivity is not as good as for precipitated samples (0.001 μ Curie will give about 200 counts/min.) in a counter of 3 cm diameter in 3 mm distance), but it is an advantage of the method that all samples always have the same size and weight with an accuracy which will not give an error larger than 1 % in the countings.

Instead of using picein for sealing the glass to the steel ring other substances can be used. Thus, HANS BOHR obtained satisfactory results by using stopcock grease for sealing one of the glasses. In that case, it is possible to clean the cuvettes by removing the glass.

I am indebted to Professor NIELS BOHR for numerous facilities put at my disposal during the work. My thanks are further due Professor G. HEVESY and cand. polyt. J. OTTESEN for helpful discussions.

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Effects of Oxygen and Carbon Dioxide on the Circulation of Isolated and Perfused Lungs of the Cat.

By

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Received 19 May 1948.

The blood vessels of the lungs are known to react on stimulation of the pulmonary vasomotor nerves, certain drugs, and the volume of the circulating blood (BRADFORD and DEAN 1889; TIGERSTEDT 1903; EULER 1932; DALY 1938; DALY, DUKE and WEATHERALL 1947). These experiments have been performed on isolated lungs as well as on intact animals.

However, factors governed by gas tensions in the blood are also important in the determination of the diameter of the blood vessels, and thereby influencing the distribution of the blood within the lungs, as was recently shown by EULER and LILJESTRAND (1946), LOGARAS (1947), and MOTLEY, COURNAND, WERKO, HIMMELSTEIN and DRESDALE (1947). These investigations have shown that increased O_2-pressure in the blood causes dilatation of the blood vessels in the lungs while contraction is a result of O_2-deficiency and increased CO_2-tension. EULER and LILJESTRAND found that these effects were not dependent on vagotomy of the animal or removal of the stellate ganglia. They concluded from this that there must exist a peripheral action unless a reflex mechanism of altogether unknown nature comes into play. LOGARAS showed that the increase of blood pressure in the pulmonary

circulation due to O_2-deficiency and increase of CO_2 was not affected by ergotamine, dihydroergotamine, atropine or yohimbine. This would seem to support the assumption of the presence of a local process.

Changes in the blood pressure in the above experiments on the cat could, however, be considered to be partly the result of changes in the blood flow through the lungs. Therefore it seemed desirable to perform experiments on isolated lungs perfused with a constant flow.

Method.

Cats weighing 1.75—3 kg were used in the experiments. The lungs were removed immediately after the animals had been anaesthetised with ether.

The lungs were perfused with a constant pulse volume and frequency using a Dale-Schuster pump, which pumped blood into the pulmonary artery through a cannula inserted in the right ventricle. The blood in the pulmonary veins was led from the left auricle to a reservoir. The aorta was closed. The perfusion blood consisted of cow's blood with the addition of heparine and diluted with one-half part Ringer's solution. The blood pressure was registered by a Hg-manometer, in some experiments by a H_2O-manometer, connected to a piston recorder. The minute volume was kept at about 30—50 cc per kg body weight and the blood pressure at about 20 mm Hg.

The lungs were placed in an air tight glass jar closed by a cork fitted with glass tubes through which the blood and gases were allowed to circulate. The trachea was connected with a Müller valve. A Starling pump rhythmically produced a negative pressure in the jar (12—16 times per min.) causing the lungs to expand and draw in the respiratory gases through the Müller valve. The jar was in contact with the surrounding air through an adjustable opening. In other experiments a positive ventilation pressure was used so that the gases were blown in through the trachea which was in connection with the surrounding air through an adjustable opening. The amount of gas inhaled with each breath could be varied in both cases by changing the size of the opening out to the surrounding air.

The glass jar containing the lungs was placed in a water bath together with the blood pump. The temperature was kept at 36—39° C.

The gas mixtures were administered through bags which were connected to the Müller valve or the Starling pump.

Results.

One rabbit and eleven cat lungs have been studied. Of these seven cat lungs were ventilated with negative pressure, the rab-

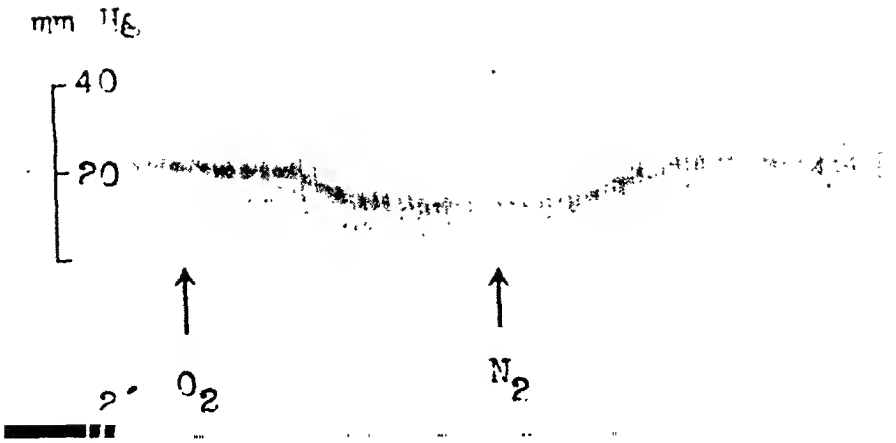

Fig. 1. Cat, 3 kg. Pulmonary arterial pressure. Negative ventilation pressure. Nitrogen inhalation at the curve's beginning. Time 2 min.

bit lung and two cat lungs with positive pressure and two cat lungs first with positive and then with negative pressure.

A. Oxygen deficiency and pure oxygen. When the lungs were alternately ventilated under negative pressure with O_2 and N_2 a marked increase of blood pressure in the a. pulm. was obtained in two preparations with N_2 (Fig. 1). The increase was in one case 10 mm Hg and in the other 4 mm Hg, corresponding to one half and one sixth respectively of the blood pressure with O_2. The changes in the blood pressure occurred over a period of 3—20 mins. probably due to the fact that the dead space was relatively large and that the blood was completely saturated with O_2 before ventilation with N_2. No change in the blood pressure of the a. pulm. was observed with alternation between N_2 and O_2 under positive ventilation pressure, even if the same preparation reacted with vasoconstriction to N_2 under negative ventilation pressure.

B. Carbon dioxide. When pure O_2 and O_2 containing 6.5 % CO_2 (carbogen) were alternated an increase in the blood pressure of the a. pulm. was obtained in 5 preparations under negative ventilation pressure and in 2 under positive pressure when carbogen was inhaled (Figs. 2, 3 and 4). The increases were from 1 to 8 mm Hg, corresponding to an increase in the latter case

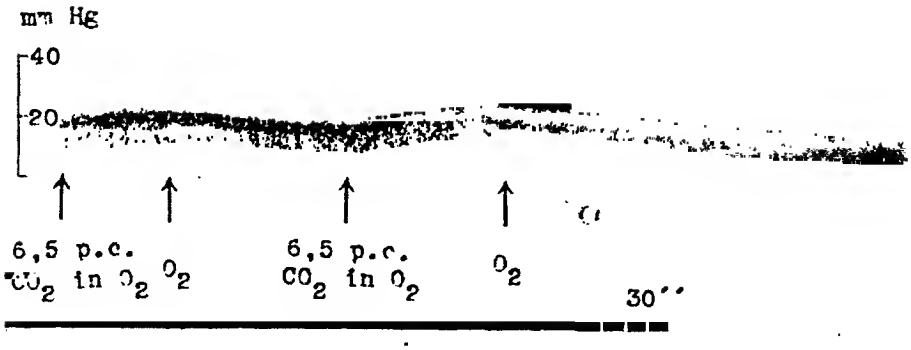

Fig. 2. Cat. 3 kg. Pulmonary arterial pressure. Negative ventilation pressure. Time 30 sec.

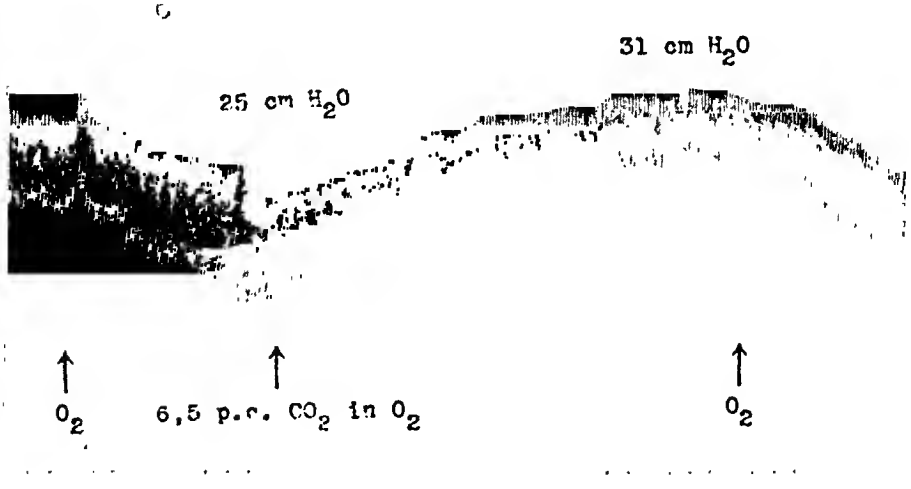

Fig. 3. Cat. 2.8 kg. Pulmonary arterial pressure. Negative ventilation pressure. 6.5 p. c. CO_2 in O_2 at the curve's beginning. Time 30 sec.

of about 40 %. Most of the preparations ventilated under positive pressure did not react with an increase in blood pressure to changes in the CO_2 content of the inhalation gas. The two, which did react, did so immediately after perfusion was started. The changes in blood pressure brought about by variations in the CO_2 content occurred more quickly than those with variations in the N_2 content.

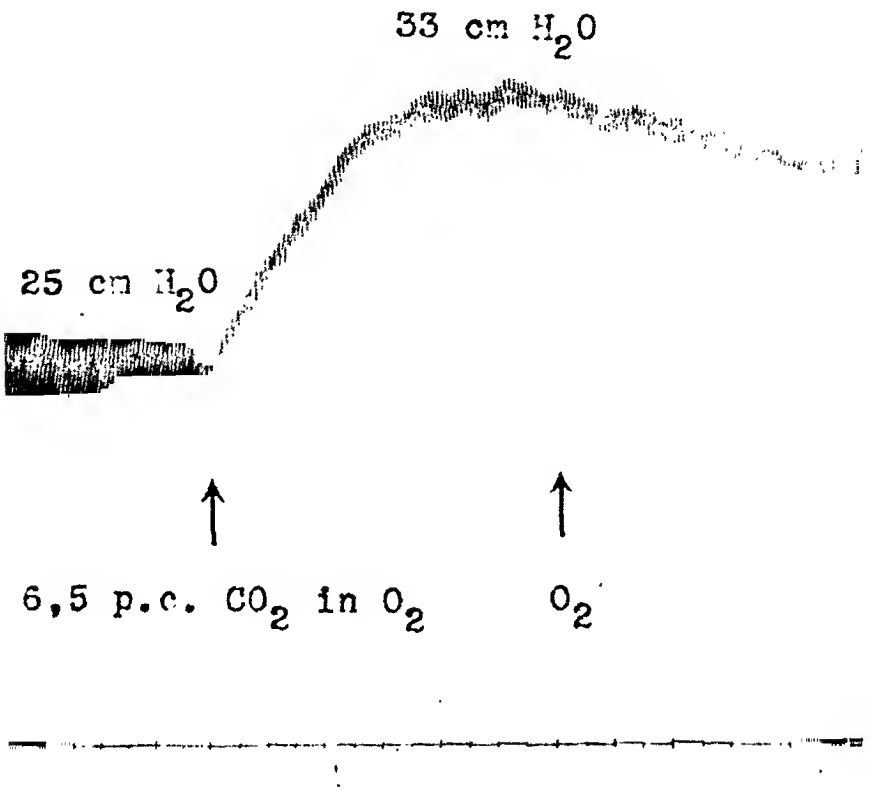

Fig. 4. Cat. 2.8 kg. Pulmonary arterial pressure. Negative ventilation pressure. O_2 at the curve's beginning. Time 30 sec.

Discussion.

It has not been definitely shown where in the path of the pulmonary blood vessels the mechanism for changes in the blood pressure are to be found.

During ventilation with air poor in oxygen, tissue hypoxia may occur, which according to Drinker should produce an increase in resistance owing to stasis and increased permeability of the pulmonary capillaries (COURNAND and associates).

According to EULER and LILJESTRAND O_2 deficiency should cause some alteration leading to a contraction of the walls of the blood vessels, thereby increasing the peripheral resistance. Consequently, if the alveolar ventilation is effective and the O_2 content is, therefore, relatively high and the CO_2 content relatively low in an area of the lung tissue, a dilating effect

is brought to bare on the arterioles, so that the blood stream increases through this area. On the other hand, with low O_2-tensions and high CO_2-tensions, that is in less well ventilated areas of the lung, the arterioles are contracted thus directing the blood to those lung areas which are best ventilated.

The effect of the inhaled gas on the resistance in the pulmonary vessels may be considered to occur either via the O_2- and CO_2-tensions of the blood or by direct diffusion through the lung tissue to the vascular wall.

The question lies near to hand whether or not an O_2 deficiency and an increased CO_2-tension causes an increase in blood pressure through some common factor, such as, change of the pH. Although a displacement of the pH in the blood can hardly be the cause since ventilation with O_2 as well as 6.5 % CO_2 in O_2 should give a more acid reaction than with N_2, the possibility is not excluded that a change can take place in the walls of the blood vessels such that both N_2 and 6.5 % CO_2 in O_2 give a more acid reaction than pure O_2 in the former case owing to hypoxia. It is, however, worth pointing out that in some cases increased CO_2-tension under positive ventilation pressure increased the blood pressure, while O_2 deficiency had no such effect.

Preparations ventilated under negative pressure showed oedema of the lungs after about 4 hours while those under positive pressure were only slightly oedematous even after 6—7 hours. This is probably due to mechanical factors (BARACH, MARTIN and ECKMAN, 1938).

Summary.

The effect of the inhalation of O_2, N_2 and 6.5 % CO_2 in O_2 was studied on isolated cat lungs perfused with a constant blood flow from a pump.

Under negative ventilation pressure an increase in blood pressure was obtained with inhalation of N_2 and a decrease with pure O_2. No changes were observed under positive ventilation pressure.

Increase of the CO_2-tension in the inhaled gas increased the blood pressure under both negative and positive ventilation pressure, although less often under positive pressure.

Lungs ventilated under negative pressure became oedematous

after about 4 hours, while those under positive pressure showed only very slight indications of oedema after 6—7 hours.

The results show that variations in the O_2- and CO_2-tensions of the inhaled gases exercise a direct effect on the pulmonary vessels.

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The Specific, Histamine Antagonizing and Non-specific, Antispasmodic Effect of Various Substances on Guinea-pig's Isolated Intestine.

By

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The modern synthetic "antihistamine drugs" or "histamine antagonists" have been widely used not only for clinical purposes in treating allergic diseases but also as a means of securing the histamine-identity of depressor and smooth muscle stimulating agents in various extracts. The exact mode of action of these antihistamine drugs is not completely known, although their capability of blocking the effects of histamine on various isolated organs as well as their power of counteracting many allergic and histamine-induced symptoms have lately been repeatedly reported.

In his comprehensive review, LOEW (1947) gives a precise definition of what should be meant with antihistamine drugs: "Those drugs which are capable of diminishing or preventing several of the pharmacological effects of histamine and which do so by a mechanism other than the production of pharmacological responses diametrically opposed to those produced by histamine may be termed *histamine antagonists* or *antihistamine drugs.*" Furthermore LOEW points out that there are a number of substances (including *e. g.* adrenaline) which, although they cause bronchodilatation, vasoconstriction, decreased capillary permeability, inhibition of intestinal activity etc. should nevertheless not be considered as true antihistamine drugs, since the

prominent effect which they induce only "represents the antithesis of those produced by histamine". Obviously a certain degree of specificity against histamine action must be attributed to the true antihistamine drug.

The isolated small intestine of guinea-pig is commonly used as test organ for the determination of minute amounts of histamine. There are, however, other substances, traces of which also induce intestinal spasm. In order to differentiate between histamine and other spasmogenic substances in various extracts the antihistamine drugs have been used by several authors, as these drugs are, even in very high dilutions, capable of abolishing the intestinal spasm induced with histamine. It has, however, been pointed out that the antihistamine drugs also counteract the effect of other spasmogenic drugs but only in doses considerably larger than those required for the antihistamine effect (HALPERN, 1942, MEIER and BUCHER, 1946, LOEW, MCMILLAN and KAISER, 1946, etc.). When using antihistamine drugs as a sort of "specific denominators" for substances of supposed histamine identity one must keep the fact in mind that the antagonizing action of these drugs is not unconditionally limited to a specific antihistamine action. In the light of previous investigations one might, however, conclude that the histamine antagonizing effect which these specific drugs exert on *e. g.* isolated intestines must be due to some quality other than ordinary antispasmodic action.

The present investigation deals with the action on isolated small intestine of guinea-pig of some commonly used antihistamine drugs, some naturally occurring but not "true" histamine antagonists such as adrenaline and nor-adrenaline (arterenol), and of some spasmolytic drugs such as papaverine and atropine. The antihistaminic effect of these substances has been compared with their antispasmodic action and the ratio between specific (histamine antagonizing) and non-specific (antispasmodic) dose has been calculated.

Methods.

I. Determination of antihistaminic and antispasmodic effect on isolated small intestine.

The isolated small intestine of guinea-pig was used as the test object. The animals were starved for about 12—15 hours before the experiment as this seemed to render the intestine more sensitive. They were then killed by a blow on the head and the blood vessels

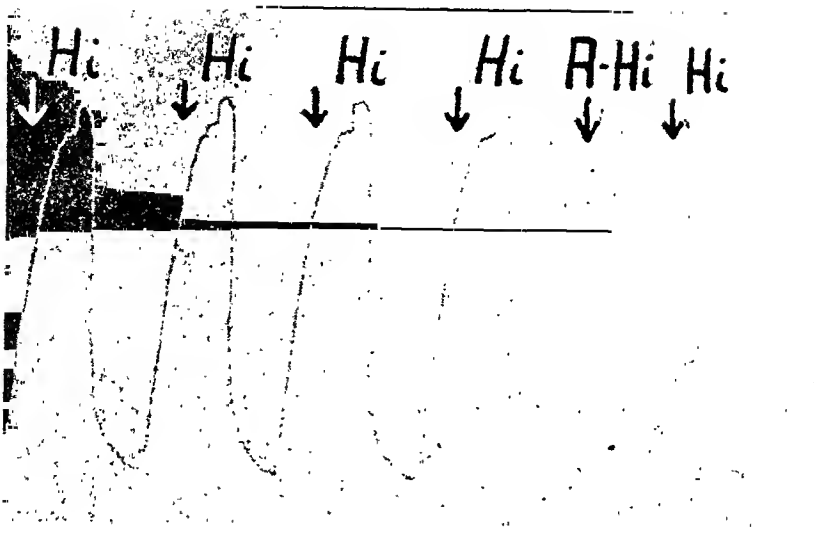

Fig. 1.

Isolated guinea-pig's intestine. Bath volume 20 ml. Hi : 0.1 μg histamine dihydrochloride (Concentration in bath: 0.005 μg/nl). AHi : 0.25 μg of an antihistamine drug (Concentration in bath: 0.0125 μg/ml). The first 4 doses of Hi give contraction heights of 40, 39, 38 and 39 mm (mean value 39 mm). After the addition of 0.25 μg antihistamine drug the same dose of Hi as before gives a contraction height of only 14 mm = 36 % of the initial value.

of the neck were opened. When the animal had finished bleeding a portion of about 30—40 cm in length of the small intestine was removed. One part of this intestine was immediately placed in luke-warm Tyrode's solution and the other part immersed in cold Tyrode's solution and stored in the refrigerator at + 2° C. After about 20 minutes the intestine placed in warm suspension fluid showed strong spontaneous movements and a piece of it was fixed in the intestinal bath, aerated by a mixture of 95 % O_2 and 5 % CO_2 ("Karbogen"). The size of the intestinal bath was 20 ml.

The portion of the intestine which was kept in the refrigerator was used the next day. About one hour before the experiment it was placed in luke-warm Tyrode's solution where it soon began to show spontaneous movements. These intestines were often even more sensitive to histamine and acetylcholine than the intestine which was used immediately after the animal had been killed. This method was employed by, amongst others, EMMELIN (1945).

In the first part of this investigation histamine dihydrochloride (Hi), acetylcholine chloride (ACh) and barium chloride ($BaCl_2$) were used in order to give contractions of the intestine. The intestines used varied somewhat in sensitivity, but usually a strong contraction was produced when the added amount of Hi and ACh gave a final concentration in the suspension fluid (Tyrode's solution) of 0.005—0.025 μg per ml. For $BaCl_2$ the necessary concentration was about 25—100 μg per ml suspension fluid. When the individual sensitivity of the intestine had been determined, a dose giving about 10—60 %

of maximal contraction was given (in conformity with the findings of EMMELIN, KAHLSON and WICKSELL, 1941). After 3 or 4 consecutive doses, giving rather constant contraction heights, the antihistamine or antispasmodic drug to be tested was added and without changing the suspension fluid the same amount as before of intestine-contracting substance (Hi, ACh or $BaCl_2$) was added (see Fig. 1). The average of the contraction heights of the preceding 3 or 4 doses was calculated and compared with the contraction height after the addition of a certain amount of antihistamine or antispasmodic drug. The remaining contraction height was expressed in percent of the initial contraction height. — When the intestine had recovered from the effect of the antihistamine or antispasmodic drug the same procedure was repeated and a higher dose of the drug was added. This procedure was repeated until the effect of the intestine-stimulating agent (Hi, ACh or $BaCl_2$) was quite abolished.

Some of the intestines were rather insensitive, giving contractions only at a concentration of 0.1—0.5 μg of Hi or ACh per ml suspension fluid. These intestines were used in order to study whether the amount of antihistamine drug which was sufficient to abolish the effect of *e. g.* 0.01 μg Hi per ml was also sufficient to block the effect of a 10—25 times greater concentration of Hi.

The composition of the Tyrode's solution which was used as suspension fluid throughout all the experiments was as follows: 0.8 % NaCl, 0.02 % KCl, 0.02 % $CaCl_2$, 0.02 % $MgCl_2 \cdot 6H_2O$, 0.1 % $NaHCO_3$, 0.005 % $NaH_2PO_4 \cdot H_2O$ and 0.1 % glucose.

The amount of histamine is expressed in μg of dihydrochloride salt and the acetylcholine in μg of chloride, since it does not seem more advantageous to express them as free base, the salts being generally used.

When the amount of antihistamine drug necessary to abolish the effects of Hi, ACh and $BaCl_2$ had been determined the action of these drugs was also tested on some other spasmogenic substances, including choline chloride, acetyl-β-methylcholine chloride, pilocarpine hydrochloride, Substance P, adenylic pyrophosphate, nicotine hydrochloride, and potassium chloride. This was done in order to elucidate further the specificity of antihistamine action.

II. The graphical representation of the results.

Since the added amounts of antihistamine and antispasmodic drugs covered a concentration range of from 0.000025 to 500 μg per ml suspension fluid it was necessary to express the concentrations as a logarithmic function of the dose. The following method was employed: The number of moles per ml of bath fluid was calculated and the logarithm of this concentration was used as a measure. Thus a concentration of *e. g.* 0.25 μg Benadryl per ml suspension fluid means $\frac{0.25}{289.5} \times 10^{-6}$ moles per ml, corresponding to the logarithmic value — 9.06.

The concentrations of the added antihistamine or antispasmodic

drug, expressed in this way, have been marked along the abscisse and on the ordinate the remaining contraction height, induced by the intestine-stimulating drugs in percent of the initial contraction height before the addition of the antihistamine or antispasmodic drug. For example, a contraction height of 100 means that the added amount of antihistamine drug does not reduce the effect of histamine at all, a contraction height of 50 means that the added amount of antihistamine drug has reduced the effect of Hi to 50 % of the initial effect of Hi etc. At 0 there is no longer any visible effect of Hi. — On the abscisse the concentration expressed as μg of antihistamine or antispasmodic drug per ml bath fluid has been marked as well, giving an easier conception of the amounts necessary to abolish the effects of Hi, ACh and $BaCl_2$.

The curves plotted in these diagrams show the relation between the concentration of antihistamine or antispasmodic drug and the blocking effect of these drugs on Hi-, ACh- and $BaCl_2$-induced intestinal contractions. The curves are, as a rule, obtaincd as mean values from 3 or 4 experiments with intestinal picces from different animals.

The antihistamine drugs used were soon found to block the effect of Hi in very low concentrations. In higher concentrations they also blocked the effect of ACh, $BaCl_2$ and other spasmogenic substances. The histamine antagonizing effect was regarded as specific, the antagonizing effect on ACh, $BaCl_2$, choline, etc., as non-specific, antispasmodic. In order to obtain a numerical expression of the relation between these two effects the *"specific: non-specific dose ratio"* has been employed. The concentration (μg/ml) of the antihistamine drug necessary to abolish the spasmogenic effect of histamine has been put equal to "1". The non-specific concentration is in this respect equal to the number of times one must increase this concentration in order to abolish also the effect of *e. g.* ACh, $BaCl_2$, etc. Thus a ratio of 1 : 400 for the action of Lergitin on ACh means that the concentration of this antihi tamine drug must be 400 times higher in order to abolish the effect of ACh than to abolish the effect of Hi (*i. e.* the specific effect).

Results.

I. Antihistamine Drugs.

The histamine antagonizing and spasmolytic effect of these drugs have been investigated by serveral authors (see LOEW's review, 1947). It is commonly stated that the different antihistamine drugs inhibit the effect of histamine on the isolated intestine in smaller concentrations than are necessary to abolish the effect of acetylcholine or barium (HALPERN, 1942; BOVET, HORCLOIS and FOURNEL, 1944; BOVET, HORCLOIS and WALTHERT, 1944; BOVET and WALTHERT, 1944; MAYER, HUTTNER and SCHOLZ,

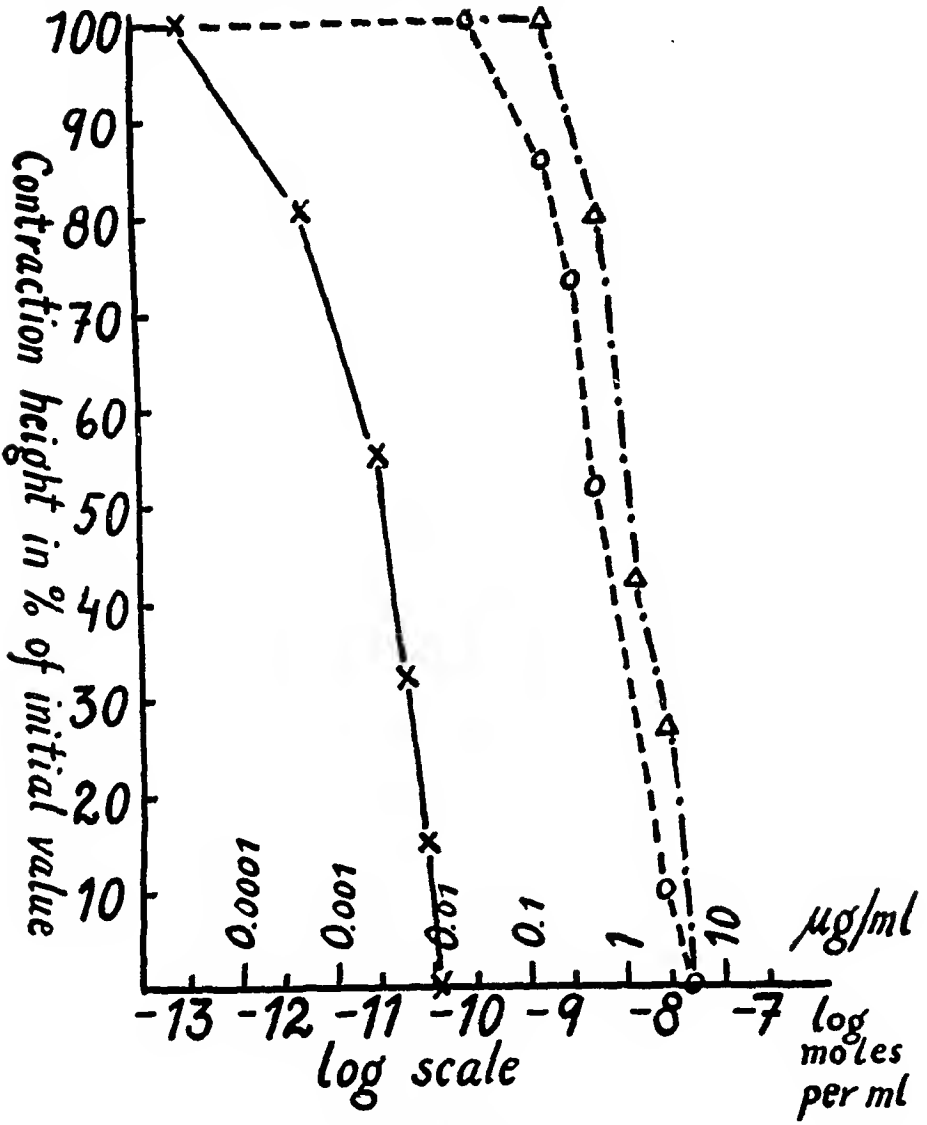

Diagram 1.

The effect of Lergitin (Antergan) on Hi, ACh and $BaCl_2$. For further explanation, see text (Methods: II. The Graphical representation of the results).

×———×———×: Histamine

○- - - -○- - - -○: Acetylcholine

△—·—·△—·—·△: $BaCl_2$

1945; MAYER, 1946; LOEW, KAISER and MOORE, 1945; LOEW, MCMILLAN and KAISER, 1946; MEIER and BUCHER, 1946 etc.).

A. N-phenyl-N-benzyl-N', N'-dimethylethylenediamine · HCl ("2339 Rhône-Poulenc", "Antergan", "Lergitin").

The preparation employed in the present investigation was

"Lergitin", the Swedish correspondence to the French "Antergan".[1]

Effect on Hi: A concentration of 0.000025 μg per ml suspension fluid did not exert any visible effect on the Hi-induced contraction. In a concentration of 0.0005 μg per ml the contraction height was reduced to approximately 80 % of the initial value and 0.0125 μg per ml inhibited the effect of Hi completely (see Diagram 1).

Effect on ACh and $BaCl_2$: In order to cause a visible decrease in contraction height the concentration of Lergitin had to be raised: for ACh to 0.05 μg per ml and for $BaCl_2$ to 0.25 μg per ml. At a concentration of 5 μg per ml suspension fluid the effect of ACh and $BaCl_2$ was abolished.

Specific : non-specific dose ratio: From the above-mentioned figures it was obvious that the histamine antagonizing effect of Lergitin appeared at a concentration when no visible effect was exerted on the intestine-contracting action of ACh and $BaCl_2$ (see Diagram 1). In order to abolish the action of ACh and $BaCl_2$ the concentration of Lergitin must be increased about 400 times (from 0.0125 μg/ml to 5 μg/ml). Thus the ratio was 1 : 400.

B. N-α-Pyridyl-N-methoxybenzyl-N', N'-dimethylethylenediamine · H_3PO_4 ("2786 R. P.", "Neoantergan").

Effect on Hi: A concentration of 0.005 μg Neoantergan per ml diminished the Hi-induced contraction to about 80 % of the initial contraction height. At a concentration of 0.0375 μg per ml the effect of the subsequent Hi was abolished.

Effect on ACh and $BaCl_2$: In the case of Neoantergan the effect of ACh and $BaCl_2$ was inhibited to the same extent, both curves following each other closely (Diagram 2). At a concentration of 1.25 μg Neoantergan per ml suspension fluid the effects of ACh and $BaCl_2$ was decreased to approximately 80 % of the initial values. Raising the concentration to about 12.5—25 μg per ml inhibited the effect of ACh and $BaCl_2$.

Specific : non-specific dose ratio: From the above-mentioned figures it follows that this ratio was, on an average, about 1 : 500, indicating a high histamine antagonizing specificity as regards the action on isolated intestinal muscle.

C. N-α-Pyridyl-N-benzyl-N', N'-dimethylethylenediamine · HCl ("Pyribenzamine").

This substance, described by Mayer, Huttner & Scholz (1945) is a homologue of Neoantergan, lacking the methoxy group

[1] AB Recip, Stockholm, kindly supplied me with the pure substance.

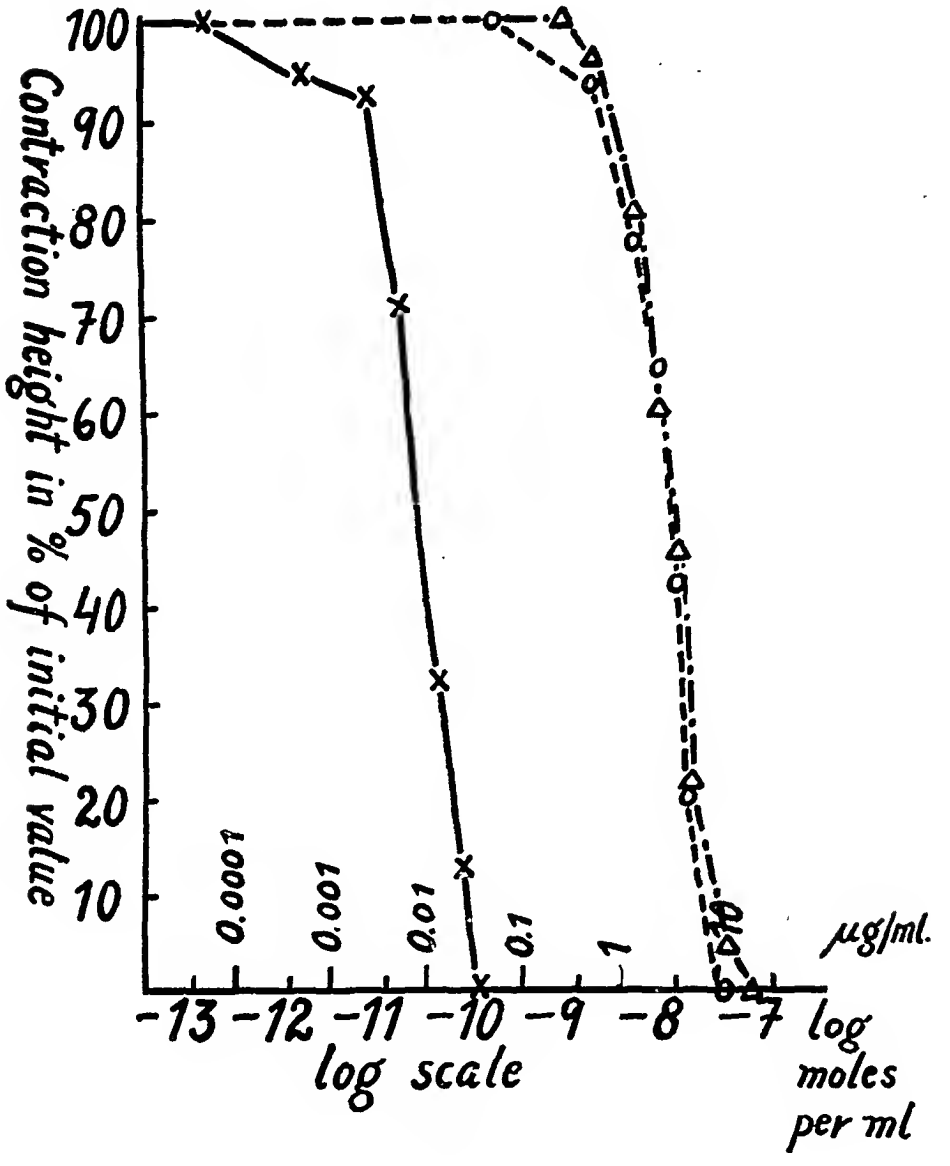

Diagram 2.
The effect of Neoantergan on Hi, ACh and $BaCl_2$.
×——×——×: Histamine
○----○----○: Acetylcholine
△·-·—·△—·—·△: $BaCl_2$

on the benzyl ring contained in Neoantergan. It could be presumed, from the close chemical relationship between Neoantergan and Pyribenzamine, that the effect of these two antihistamine drugs should be, as a matter of fact, rather identical. In view of the present findings this is, however, not entirely the case.

Effect on Hi: A decrease of the Hi-induced contraction to about 65 % of the initial height was observed when the amount of Pyribenzamine added gave a final concentration in the intestinal bath of 0.0005 μg per ml. The Hi-induced contraction was quite abolished when this concentration was raised to 0.0125 μg per ml. These figures are not the same as those for Neoantergan and when comparing the histamine antagonizing effect of these two drugs Pyribenzamine seems to have a somewhat stronger antihistamine action (cf. Diagram 3).

Effect on ACh and $BaCl_2$: The close parallelism between the inhibiting action of Neoantergan on ACh- and $BaCl_2$-induced contractions was not found in the case of Pyribenzamine. In order to annul the effect of ACh a concentration of 12.5 μg Pyribenzamine per ml suspension fluid was needed, the corresponding figure for $BaCl_2$ being as high as 50 μg per ml.

Specific : non-specific dose ratio: Because of the discrepancy between the antispasmodic effects on ACh and $BaCl_2$ respectively it seems essential to distinguish between a Hi : ACh ratio and a Hi : $BaCl_2$ ratio, the former being 1 : 1,000, the latter being 1 : 4,000.

D. β-Dimethylaminoethyl benzhydryl ether · HCl ("Benadryl", "Deseryl").[1]

Effect on Hi: In a concentration of 0.0005 μg Benadryl per ml suspension fluid the effect of Hi was reduced to 90 % of the initial value and a concentration of 0.025 μg per ml abolished the effect of the subsequent Hi-dose.

Effect on ACh and $BaCl_2$: A concentration of 0.5 μg Benadryl per ml was necessary to abolish the effect of ACh and 1.25 μg per ml was needed to reduce the effect of the subsequent $BaCl_2$-dose to nothing. It was obvious that the antispasmodic, non-specific effect of Benadryl is somewhat different as regards the two substances ACh and $BaCl_2$, the effect of the first one of these two spasmogenic drugs being more easily blocked.

Specific : non-specific dose ratio: According to the above-mentioned figures this ratio was on an average (mean values of ACh- and $BaCl_2$-blocking concentrations) 1 : 35, indicating a rather weak histamine antagonizing specificity as regards the action on isolated intestine.

E. 2-N-Phenyl-N-benzylaminomethylimidazoline ("Antistine", "Antasten").

Effect on Hi: In a concentration of 0.0005 μg Antistine per ml

[1] Parke, Davis & Co kindly supplied me with the pure substance.

suspension fluid the Hi-induced intestinal contraction was reduced to about 90 % of the initial height. A concentration of 0.25 μg per ml was necessary to abolish totally the effect of a subsequent Hi-dose.

Effect on ACh and $BaCl_2$: A concentration of 2.5—5 μg Antistine per ml suspension fluid reduced the effect of ACh and $BaCl_2$ to about 60 % of the initial value. The blocking dose was, for ACh 25 μg and for $BaCl_2$ 75 μg Antistine per ml.

Specific : non-specific dose ratio was in the case of Antistine on an average 1 : 200.

F. A comparison between the antihistamine drugs tested.

A comparison between the different antihistamine drugs used in this investigation showed that their histamine antagonizing power is of about the same magnitude (see Diagram 3), although certain differences in specific activity exist. Taking into consideration only the amounts necessary to abolish totally the effect of a subsequent Hi-dose the following succession as regards the histamine antagonizing action on isolated intestine was found:

Antihistamine drug	Concentration in µg/ml	Concentration expressed as log moles per ml
Pyribenzamine	0.0125	— 10.37
Lergitin (Antergan)	0.0125	— 10.36
Benadryl	0.025	— 10.06
Neoantergan	0.0375	— 10.01
Antistine	0.25	— 9.08

When comparing the specific : non-specific dose ratio the following succession was obtained (see table below). In this table a differentiation has also been made between the Hi : ACh and Hi : $BaCl_2$ ratio.

Antihistamine drug	Specific : non-specific dose ratio	
	Hi : ACh	Hi : $BaCl_2$
Pyribenzamine	1 : 1,000	1 : 4,000
Lergitin	1 : 400	1 : 400
Neoantergan	1 : 330	1 : 670
Antistine	1 : 100	1 : 300
Benadryl	1 : 20	1 : 50

G. The relation between the histamine dose and the amount of antihistamine drug necessary to block it.

Using intestines with different sensitivity it was possible to

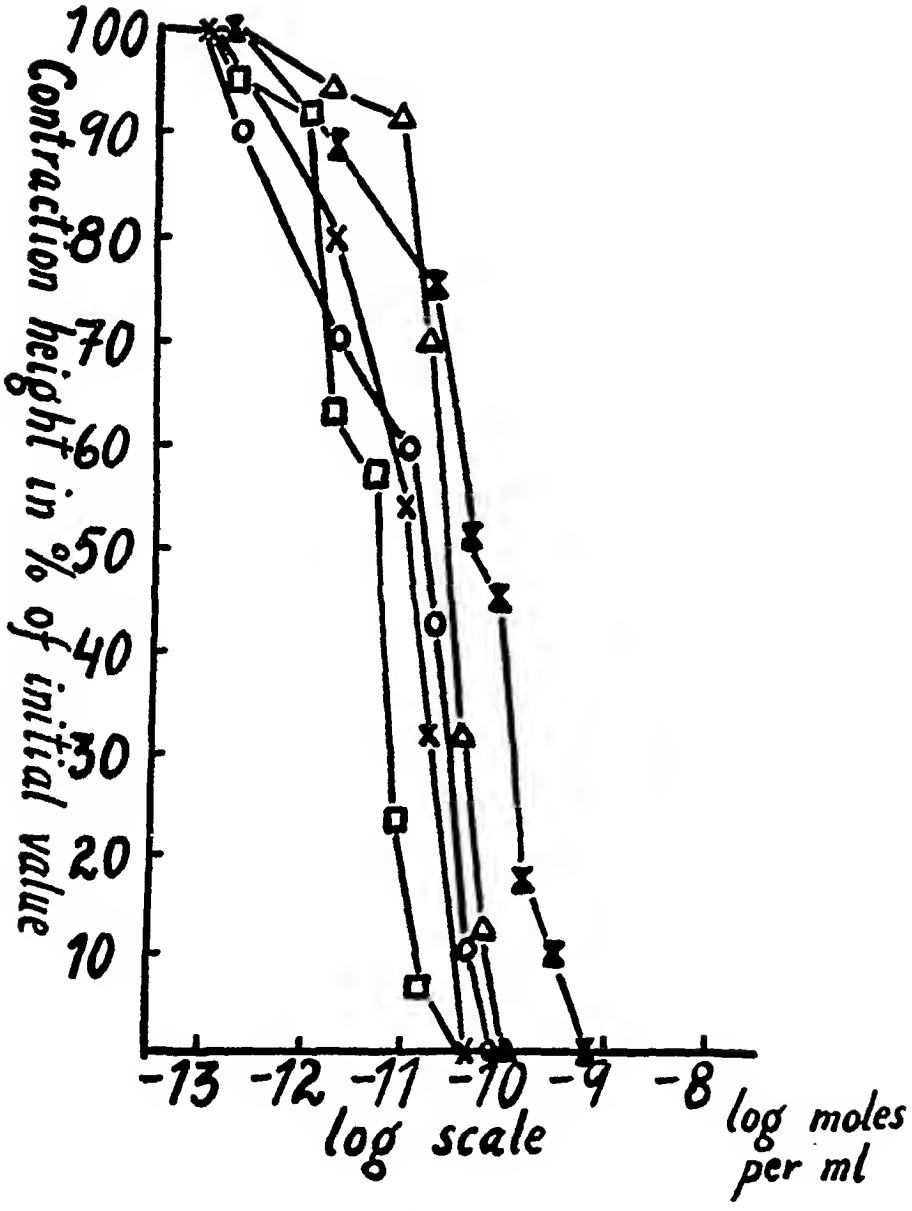

Diagram 3.

A comparison between the histamine antagonizing effect of the antihistamine drugs used.

×——×——× : Lergitin
○——○——○ : Benadryl
□——□——□ : Pyribenzamine
⧗——⧗——⧗ : Antistine
△——△——△ : Neoantergan

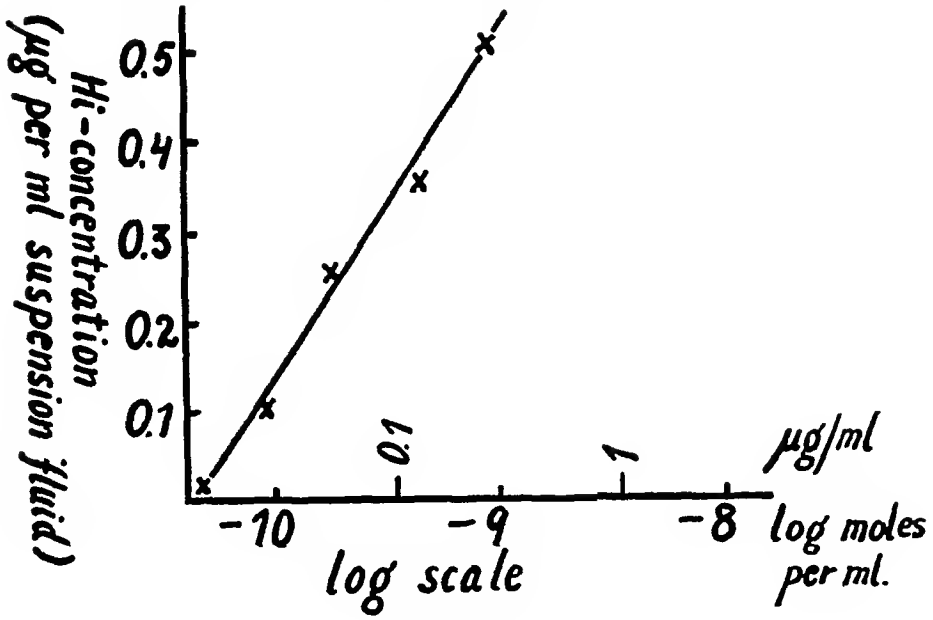

Diagram 4.

The relationship between different Hi-concentrations in the suspension fluid (giving approximately the same contraction heights — different sensitivity of the intestines) and the concentration of antihistamine drug (Lergitin) necessary to abolish totally the effect of these Hi-concentrations.

study if the added amount of antihistamine drug was able to antagonize not only a certain dose of Hi but also to block the effect of higher doses of Hi, giving, on insensitive intestines, about the same initial contraction height (equi-active doses).

Judging from experiments with Lergitin and Pyribenzamine it became evident that the amount of antihistamine drug, necessary to inhibit the effect of *e. g.* 0.01 µg Hi per ml suspension fluid was not sufficient to abolish also the effect of *e. g.* 0.25 µg Hi per ml (see Diagram 4). Obviously a certain amount of antihistamine drug did not "cover" more than a certain amount of Hi, even if the contraction height was the same for both Hi-doses. This shows that the effect of the antihistamine drug is not due to a complete blocking of the "effector cells" against any further influence of Hi. This fact was further established through experiments where, after the addition of a Lergitin-dose, sufficient to abolish totally the effect of a subsequent dose of *e. g.* 0.01 µg Hi per ml, the Hi-dose was raised 5—10 times. The contracting effect of Hi then became visible again.

It was sometimes noted that the successive addition of increasing amounts of antihistamine drugs caused a slight sensitiza-

tion against the drug. Thus if the test procedure had proceeded so far that the effect of the added Hi-dose was quite inhibited by a certain amount of antihistamine drug, it was found that when a new piece of the same intestine was put in the intestinal bath the same amount of antihistamine drug was no longer sufficient to inhibit totally the effect of the Hi-dose used. A small contraction was now seen which, however, never amounted to more than about 5—10 % of the initial contraction value.

H. The action of the antihistamine drugs on other spasmogenic substances.

From the above-mentioned results it seems obvious that there exists a distinct difference between the histamine antagonizing effect and the antispasmodic effect of the employed antihistamine drugs, a much higher concentration being needed to abolish the effects of ACh and $BaCl_2$ than to abolish the effect of Hi.

It was furthermore studied if this specificity holds true also when comparing the effect on Hi and on some other intestine contracting drugs, such as choline chloride, acetyl-β-methylcholine chloride ("Mecholyl"), pilocarpine hydrochloride, Substance P (EULER and GADDUM),[1] adenylic pyrophosphate, nicotine hydrochloride and potassium chloride. The effect of antihistamine drugs on intestinal contractions, caused by an increase of pH in the suspension fluid, was also tested.

It was found that none of these intestine contracting substances was antagonized by the antihistamine drugs in concentrations which abolished the effect of equi-active doses of Hi but only in higher, antispasmodic concentrations.

The following table shows the specific : non-specific dose ratio for the antihistamine drugs used as regards the above-mentioned spasmogenic substances:

	Lergitin	Pyri-benzamine	Neoantergan	Antistine	Benadryl
Hi	1	1	1	1	1
ACh	1 : 400	1 : 1,000	1 : 330	1 : 100	1 : 20
$BaCl_2$	1 : 400	1 : 4,000	1 : 670	1 : 300	1 : 50
Choline	1 : 400	1 : 1,000	1 : 330	1 : > 200	1 : 20
KCl	1 : 2,000	1 : > 1,000	1 : 670	1 : > 100	1 : 1,000
Pilocarpine	1 : 200	1 : 200	1 : > 67	1 : 100	1 : > 10
Substance P	1 : > 400	1 : > 200	1 : 670	1 : > 100	1 : > 200
Mecholyl	1 : 400	1 : 1,000	1 : 670	1 : 100	1 : 20
Nicotine	1 : 400	1 : 1,000	1 : 330	1 : 50	1 : 50

[1] Prof. U. S. v. EULER kindly supplied me with a preparation of this substance.

The determination of a specific: non-specific dose ratio for the antihistamine drugs as regards their action on *adenylic pyrophosphate* was found rather difficult, since the successive addition of this substance rapidly destroyed the sensitivity of the intestines. It was, however, stated that the doses of the antihistamine drugs which abolished the effect of Hi did not cause any visible decrease in the contractions caused by adenylic pyrophosphate.

The constant high figures for KCl are probably due to the fact that the contraction caused by KCl was only partly due to the effect of the potassium ion, since it was found that the addition to the intestinal bath of a corresponding dose of NaCl also caused a slight but quite visible contraction of the intestine, obviously due to osmotic phenomena.

In the case of Substance P it was found that the effect of this intestine stimulating preparation was diminished to about 50 % when adding small, Hi-specific doses of the antihistamine drugs. After this initial reduction the effect of the Substance P-preparation employed persisted even after the addition of rather large, "non-specific" amounts of antihistamine drugs.

By raising the pH of the suspension fluid a contraction of the intestines was obtained. This contraction could be characterized as a slow, but strong increase in the intestinal tone and thus differed from the usual appearance — the sharp rise caused by spasmogenic substances. The Tyrode's solution employed had a pH of, on an average, 7.8. When adding 0.025—0.1 ml (1—4 drops) of 1-N NaOH the pH rose to 8.9—9.5 and this was sufficient to cause an increase in tone. This contraction could be counteracted by means of the antihistamine drugs but only in high, "non-specific" concentrations.

II. Adrenaline and Nor-adrenaline (Arterenol).

Adrenaline induces effects which "represent the antithesis of those produced by histamine" and should therefore, according to LOEW's definition not be looked upon as a true antihistamine drug. It seemed worth while, however, to investigate to what extent adrenaline is capable of blocking the effects of Hi, ACh and $BaCl_2$ on the isolated small intestine. At the same time it could be of a special interest to study the effect of nor-adrenaline, the non-methylated adrenaline compound which is known to mimic the effects of sympathetic excitatory impulses and to be, with a

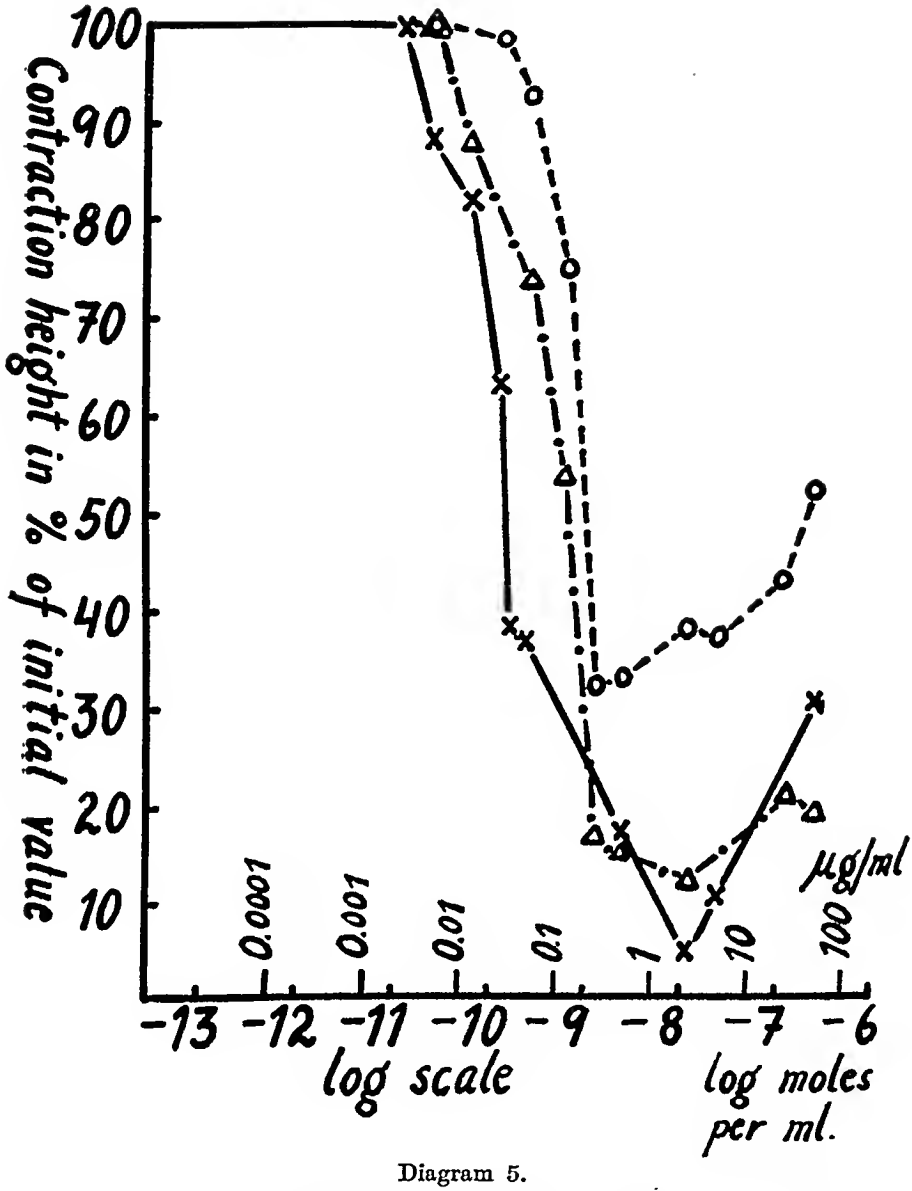

Diagram 5.

The effect of d,l-nor-adrenaline (arterenol) on Hi, ACh and $BaCl_2$.
×———×———×: Histamine
○----○----○: Acetylcholine
△—·—·△—·—·△: $BaCl_2$

certain degree of probability, identical with the sympathin E substance, postulated by CANNON and ROSENBLUETH (BARGER & DALE, 1910, BACQ, 1934, VON EULER, 1945—46, BACQ & FISCHER, 1947, GADDUM & GOODWIN, 1947, SCHMITERLÖW, 1948 etc.). It could be anticipated that nor-adrenaline should not exert the same inhibitory action as adrenaline.

A. Adrenaline.

Effect on Hi, ACh and $BaCl_2$: Adrenaline in rather high concentrations inhibited the effect of Hi, ACh and $BaCl_2$. A dose of 50 μg adrenaline per ml suspension fluid was necessary to abolish the effect of the subsequent Hi-dose, the corresponding figures for ACh and $BaCl_2$ being somewhat lower, viz. 1.25 and 25 μg respectively.

B. d,l-Nor-adrenaline (arterenol).

Effect on Hi, ACh and $BaCl_2$: Nor-adrenaline was not capable of abolishing totally the effects of Hi, ACh and $BaCl_2$, the following phenomenon being constantly observed: With increasing doses of nor-adrenaline there was at first a decrease in the response to Hi, ACh and $BaCl_2$. This decrease closely paralleled the inhibiting effect of adrenaline. A further increase of the added amounts of nor-adrenaline did not, however, reduce the contractions to nil but on the contrary caused a rise in the curves (see Diagram 5). This effect was most pronounced as regards the action of ACh.

III. Papaverine and Atropine.

In order to compare the effects of antihistamine drugs with a pure spasmolytic agent, the effect of papaverine hydrochloride has been tested in the same way. Furthermore the effect of the specific "anti-acetylcholine" drug atropine, was tested.

Papaverine inhibited the effects of Hi, ACh and $BaCl_2$ equally, the three curves running close together (see Diagram 6). The antispasmodic effect was total when the added amount of papaverine gave a final concentration of, on an average, 62.5 μg per ml suspension fluid.

Atropine differed in antispasmodic action. As could be expected, the inhibiting effect on ACh was the most pronounced, a concentration of 0.0375 μg atropine sulphate per ml suspension fluid being sufficient to annul the effect of the subsequent ACh-dose. The effect of Hi was abolished at a concentration of 2.5 μg atropine per ml. $BaCl_2$ was not quite abolished even when the added amount of atropine was as high as 10 mg (giving a final concentration of 500 μg per ml suspension fluid). Obviously the anti-ACh-action of atropine in comparison with its general spasmodic action is fairly specific. (Diagram 7).

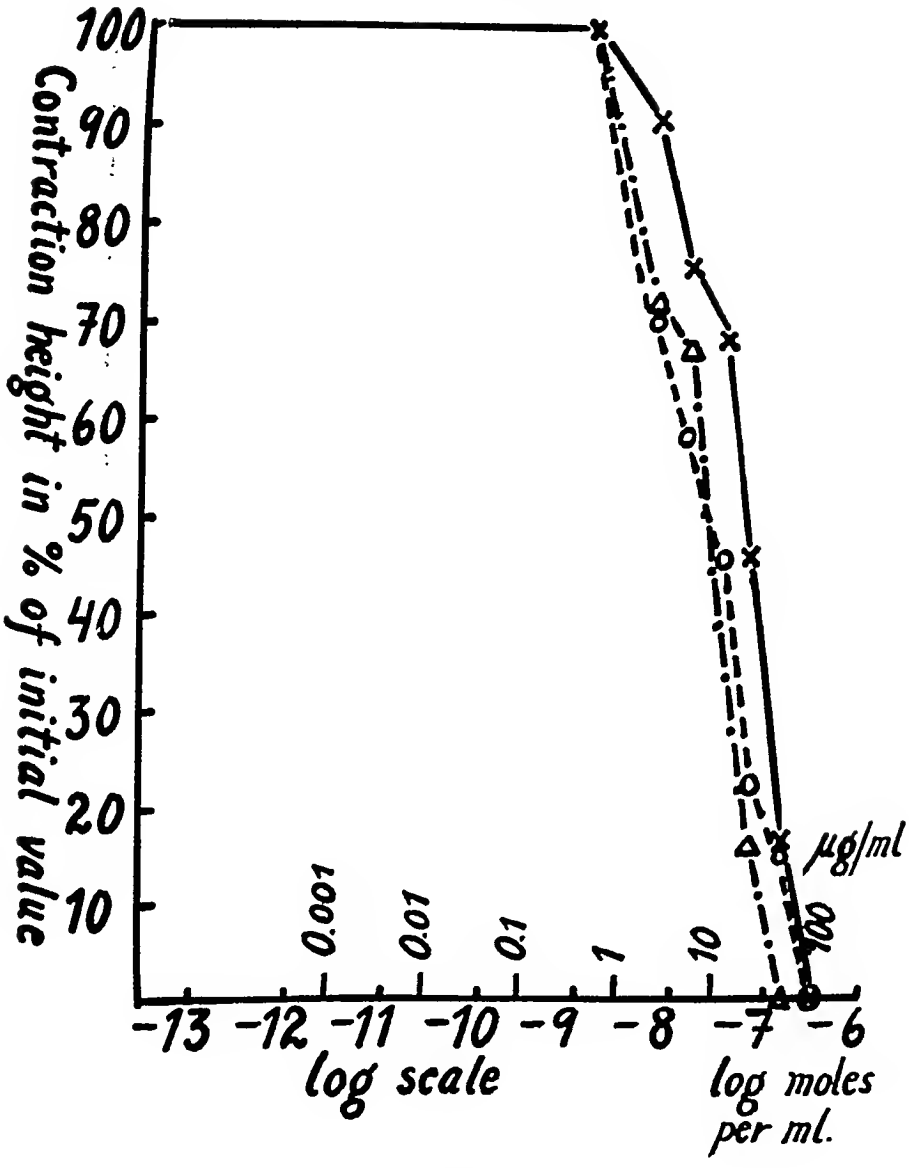

Diagram 6.
The effect of papaverine on Hi, ACh and $BaCl_2$.
×——×——×: Histamine
○----○----○: Acetylcholine
△—·—·△—·—·△: $BaCl_2$

Discussion.

The antihistamine drugs counteract in some way or other the pharmacological effects of histamine. This action is rather general and also supposed to be fairly specific. For that reason these drugs

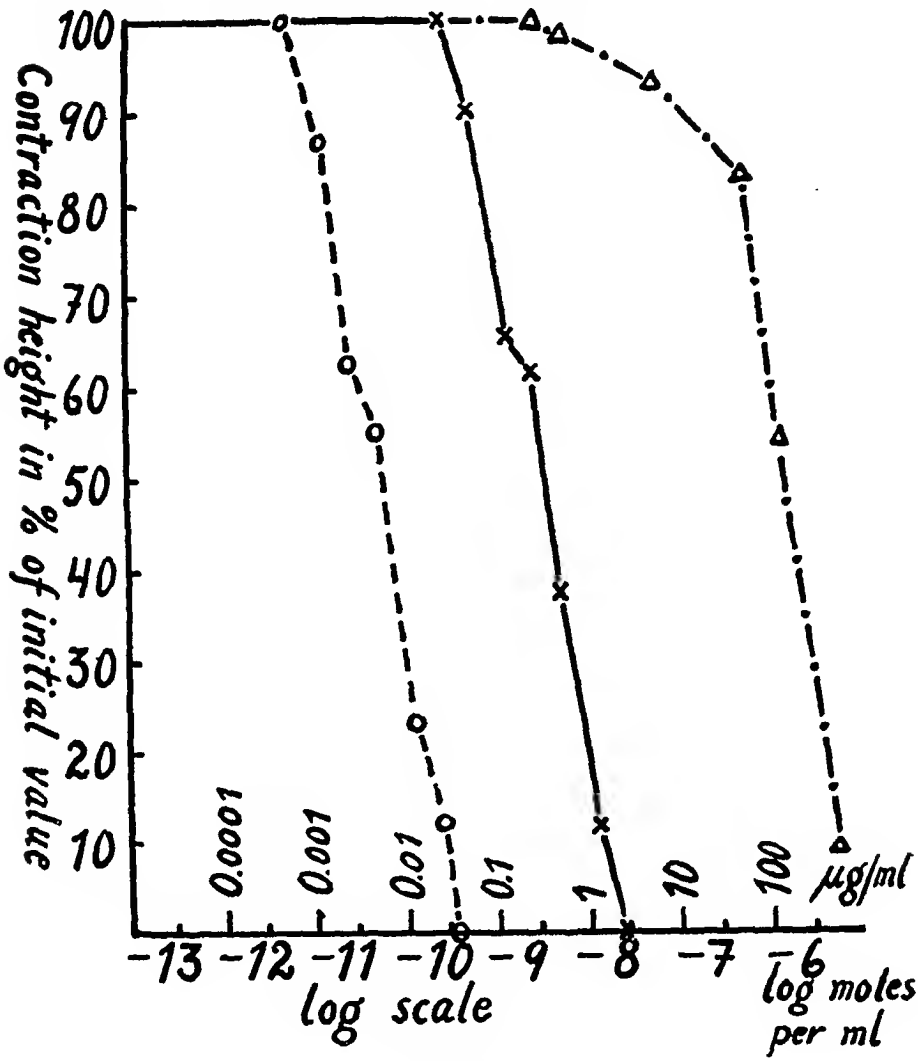

Diagram 7.
The effect of atropine on Hi, ACh and $BaCl_2$.
×———×———×: Histamine
○----○----○: Acetylcholine
△—·—·△—·—·△: $BaCl_2$

have, apart from their clinical use as more or less potent anti-allergic drugs, also been used in order to secure the identity of substances which are suspected to be histamine.

Extracts from different organs and tissues, including blood, are frequently found to contain amongst others a depressor and smooth muscle stimulating substance (or substances). By different methods it is often possible to separate this substance from the rest of the extract and it is then valuable to know if this depressor

substance is identical with histamine, since the contamination with even small amounts of this substance may cause widespread pharmacological actions. In other cases different organs are extracted with the intention of determining their content of histamine. In these cases also it is necessary to secure the histamine-identity. For these purposes the antihistamine drugs have been used and it is then presumed that if the effect of the histamine-supposed substance is abolished after the addition of an antihistamine drug its identity is secured.

The present investigation deals with the specificity of the histamine antagonizing action only as regards the effect on isolated small intestine since the usual and most sensitive method of determining histamine is the biological test on guinea-pig's isolated small intestine. The results show that the antihistamine drugs which have been tested might be used for the pharmacological differentiation between histamine and other smooth muscle stimulating agents, but only under certain conditions. The specific (histamine antagonizing) : non-specific (antispasmodic) dose ratio must always be kept in mind. When too much of an antihistamine drug is added to the intestinal bath, not only histamine, but also acetylcholine, choline, KCl, Substance P, adenylic pyrophosphate etc. will be counteracted in a non-specific way. In order to secure the histamine-identity the amount of antihistamine drug added must not exceed certain values, determined in this investigation.

Furthermore, it must be taken into consideration that the amount of the antihistamine drug necessary to abolish the effect of histamine on isolated small intestine is dependent also on the amount of histamine added. A high dose of histamine is not counteracted by the same amount of antihistamine drug which is capable of blocking the effect of a small amount of histamine, there being a distinct correlation between antihistamine action and histamine dose. The "effector cells" — in this case the smooth muscles of the intestinal wall — are not indefinitely blocked against the action of histamine by the addition of antihistamine drugs since an increase of the added histamine dose will still evoke a contraction.

The histamine antagonizing action of the antihistamine drugs is probably not only quantitatively but also qualitatively different from the antispasmodic action which they exert in higher concentrations. Using an ordinary spasmolytic drug, such as pa-

paverine, it will be noticed (Diagram 6) that the antispasmodic effects on spasms induced by histamine, acetylcholine and barium are very similar, the three curves running close together. Obviously histamine is not generally more easily blocked by antispasmodic drugs than is *e. g.* acetylcholine. The antihistamine drugs, on the other hand, block the effect of histamine on the isolated intestine already in concentrations where no effect is seen on acetylcholine, barium, choline, Substance P, etc. This fact strongly suggests that the histamine antagonizing effect is not due to a special histamine antispasmodic action but to some other, specific "histamine inactivating" effect. The same sort of specific action is observed when testing the antispasmodic action of atropine on histamine, acetylcholine and barium. In this case the effect against acetylcholine is of quite another potency than the effect against *e. g.* histamine. It is well known that atropine relaxes intestinal spasm when due to parasympathetic stimulation (or acetylcholine) but that it is not very potent against other intestinal stimulation. Judging from analogy it might then be supposed that the action of the antihistamine drugs on histamine could be in some way similar to the action of atropine on parasympathetic stimulation and acetylcholine, which would open new aspects concerning the mode of action of histamine. WELLS, MORRIS, BULL and DRAGSTEDT (1945), working with Benadryl and studying its antagonizing action on the blood pressure lowering effect of histamine, suggested that the antihistamine action might be due to some sort of competition between histamine and Benadryl for a given site of action or receptive substance. If Benadryl combines with the receptive substance no particular reaction occurs, but it prevents, thereby, histamine from combining with this same site with the production of a biological effect. These authors also point out a presumed parallelism between the system atropine—acetylcholine and the system antihistamine drugs—histamine.

As could be expected adrenaline exerts a stronger action against acetylcholine than against histamine. This also supports the fact that the effect of histamine is not in itself more easily counteracted by substances which could be regarded as physiological histamine antagonists although they are, by definition, excluded from the group of true antihistamine drugs. This favours the view that the specific action of antihistamine drugs is not only quantitatively but also qualitatively different from the antispasmodic action which they exert in higher concentrations.

Nor-adrenaline in small doses decreases the activity of histamine and other spasmogenic drugs but is, however, not able to abolish totally the effect of these intestine-contracting substances, which is in accordance with the view that nor-adrenaline mainly mimics the action of excitatory sympathetic impulses.

Summary.

Using guinea-pig's isolated small intestine as test organ the effects of five antihistamine drugs, viz. Lergitin (Antergan), Neoantergan, Pyribenzamine, Benadryl, and Antistine have been tested on spasms induced by histamine, acetylcholine, barium, choline, acetyl-β-methylcholine, pilocarpine, Substance P, adenylic pyrophosphate, nicotine, potassium, and contractions due to a change of the pH of the suspension fluid. The effects of adrenaline, nor-adrenaline, papaverine, and atropine on histamine, acetylcholine and barium have been tested as well.

The antihistamine drugs used have a specific, histamine antagonizing, and a non-specific, antispasmodic effect. The relation between the dose necessary to abolish the effect of histamine and the dose necessary to abolish the effects of the other spasmogenic drugs have been calculated. The specific : non-specific dose ratio varies for the antihistamine drugs used, Pyribenzamine, Lergitin (Antergan) and Neoantergan being the most specific ones.

Acetylcholine is antagonized by adrenaline in lower concentrations than are needed to abolish the effects of histamine and barium. Nor-adrenaline is not capable of abolishing totally the effects of these spasmogenic substances.

Papaverine antagonizes the effects of histamine, acetylcholine and barium equally. The effect of acetylcholine is blocked by atropine in a concentration when no effect is exerted on the spasmogenic actions of histamine and barium. The action of the antihistamine drugs against histamine has been compared with this specific effect of atropine against acetylcholine.

In order to secure the identity of histamine-supposed substances it is necessary to keep the fact in mind that the antihistamine drugs in higher concentrations also abolish the effects of spasmogenic substances, other than histamine. The amount of antihistamine drug used for this "identity-test" must not exceed certain values, determined in this investigation. It is also necessary to remember the fact that high doses of histamine are not coun-

teracted by the same amount of antihistamine drugs as low histamine doses. There is a definite relation between the dose of histamine and the dose of antihistamine drug necessary to abolish it.

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A Humoral Mechanism in Anoxic Erythrocytosis.

By

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The question of the effect of anoxia upon the red blood picture was raised by the statement that during a sojourn at high altitudes the red blood cells and haemoglobin of men and test animals were increased (BERT 1882, VIAULT 1890, BÜRKER 1904, HALL, DILL and BARRON 1936 etc.). Attempts were made to discover the chief erythropoietic factor in the high mountain air. As, in general, a lack of oxygen in the bone marrow, due *e. g.* to sclerosis of the vessels (the disease of VAQUES-OSLER), vasoconstriction caused by ephedrin (DAVIS, 1941) or to lowered oxygen tension of the blood, is known to cause erythrocytosis, the decreased partial pressure of oxygen in the inspired air and consequently in the arterial blood is considered to be the main factor. It seems, however, not to be the only one. According to KESTNER (1921), SEYDERHELM (1932), and BOCKSTRAHLER (1936) ultraviolet radiation is another important erythropoietic agent.

The mechanism responsible for the anoxic polycythaemia has been discussed by several authors, but still remains an unsolved question. According to BÜRKER and collaborators (1913) the *early* increase of the red cell count which takes place immediately after arriving in high altitudes, is due to an emptying of blood depots. This increase will later, in the 2nd and 3rd week, be replaced by an increased erythropoiesis in the red bone marrow. ASMUSSEN and NIELSEN (1945) claim that the initial increase of the red blood cell count is due to a loss of fluid through

the capillaries which results in haemoconcentration. The early erythrocytosis would therefore be only an *apparent* increase in the cell count. The occurrence of reticulocyte responses in low pressure stated by GERKE (1935), KRUPSKI and ALMASY (1937), GORDON and KLEINBERG (1937), seems, however, to indicate an increased output of red cells from the bone marrow. Studies on the osmotic resistance of red cells after a sojourn in high mountains seems to confirm the opinion regarding the fairly late changes in the erythrocyte count (WILBRANDT and HERMANN, 1944). GORDON and KLEINBERG arrive at the conclusion that the exposure to low athmospheric pressure stimulates the haemopoietic as well as the haemolytic mechanisms, and VERZÁR and VÖGTLI (1945), and OUJANSKY (1945) consider the two processes interrelated, in so far as an initial increased haemolysis gives rise to the formation of new erythrocytes in the bone marrow. The factor responsible for the polycythaemia would, according to the authors, probably be the bilirubin formed from the haemoglobin, or, possibly, the iron liberated thereby. GIANNINI (1929) has in fact observed bilirubinaemia in animals after exposure to low athmospheric pressure, and VERZÁR and ZIH 1929, and ZIH (1930) stated a definite erythropoietic effect with bilirubin and other haemoglobin derivatives. On the other hand LOESCHKE and SCHWARZER (1939) were not able to produce erythrocytosis in test animals on injection of icteric sera from young babies. If, however, some chemical compound formed during the anoxia or by the destruction of red cells were the physiological stimulus of the bone marrow, it would be possible to transfer the erythrocytosis producing effect of low oxygen pressure by injecting serum from the exposed animal to one which is not suffering from anoxia.

Experiments carried out in order to state such a humoral transferability of the erythropoietic effect of anoxia date back to the first and second decades of this century. CARNOT and DEFLANDRE (1906), GIBELLI (1911), MÜLLER (1912) (see also WESTPHAL, 1944) have stated erythropoietic substances in the serum of anaemic animals, active when injected into normal animals. Later authors claim on the other hand that *no erythrocytosis* can be found in *normal* animals when injected with the serum from anaemic animals (FEEDENS, 1936, GORDON and DUBIN, 1934) or when injected with the serum of animals kept under low athmospheric pressure (FOERSTER 1932, GORDON and DUBIN 1934). When, however, the test rabbits are made anaemic, *e. g.*

by bleeding, and the recovery closely observed, it can be stated that while normal serum has no effect, the injection of serum from a rabbit kept under low pressure, shortens the time of recovery (FOERSTER, KRÄHENBÜHL 1933, GABATHULER 1929). OUJANSKY reports, that the serum from anaemic animals may cause either erythrocytosis or erythropenia when injected into normal animals.

LOESCHKE and SCHWARZER (1939) compare the fetal conditions to those at high altitudes. They claim that before acclimatization has taken place, the serum of men staying at high altitudes, as well as the serum obtained from the umbilical cord of a fetus, or from infants during the first week of life, causes erythrocytosis when injected into normal rabbits. Only a few experiments were, however, made.

The erythropoietic substances contained in serum have been closely studied by TÊI YU-TIN (1938). (As the original papers have not been accessible to us no information as to the methods used or to the extent of the experimental work has been available). According to the author, erythropoietins which are highly active when injected into normal animals, are present in the serum of rabbits rendered polycythaemic by exposure to low pressure and in that of rabbits made anaemic in different ways: *e. g.* by bleeding, garlick-feeding, phenylhydrazin-poisoning, excessive ultraviolet irradiation and blocking of the RES. These active substances differ chemically from each other.

Our present knowledge of the formation of erythropoietins and their rôle in anoxic conditions is limited. The results regarding the humoral transferability are rather confusing. As a rule only anaemic animals react to the injections; normal animals generally give no response. If the substances formed under anoxia are of some importance in the regulation of the erythropoiesis during oxygen want, they should, of course, affect a normal organism as well as an anaemic. On the other hand, samples which can be injected into the recipient animal cannot be very large and in consequence the effect cannot be expected to be great. The only way to prove that there are active substances is therefore to carry out the experiments on a sufficiently large scale and to apply statistical methods to the analysis of the results. The purpose of this paper is to report on erythrocytosis promoting agents in the plasma of patients with chronic stagnant anoxia (congestive heart failure) and in acutely anoxic rabbits (exposure

to lowered athmospheric pressure). The physiological properties of the erythropoietic factors will be treated later, in separate papers.

Material and Methods.

Adult rabbits, weighing 2—3.5 kg kept on a diet of oats, hay, and swedes, were used as test animals. The weight of the test animals was controlled the same day as the blood samples were taken. Before each experiment the red blood cells were enumerated and the haemoglobin determined at intervals of 2—3 days until adaptation to the experimental conditions had taken place and a fair constancy of the red blood picture had been obtained.

The blood samples for determination of the red cells and the haemoglobin were taken between 9 and 11 a. m. The ear vein was pricked with a needle and the drop of blood formed was drawn into a heparinized, empty syringe. For each determination 0.3—0.5 cc. of blood were taken. For the red cell counts a Buerker counting chamber was used and in each determination 8 or 16 squares were counted. During the early part of this work the assistant performing the count lacked sufficient training and on that account complete double determinations were made. The mean difference between the double determinations was 180,000 cells. In the latter part, comprising all the low pressure experiments and the corresponding control experiments, the red cells were counted by a more skilled person and the mean difference between the double determinations was then only 60,000 cells. As this was considered good accuracy double determinations were not made during the last experiments. The determinations of the haemoglobin percentage were made with a Hellige-Sahli-haemometer. There was good correspondence between the paired determinations, the average difference being 1.1 %.

For the reticulocyte count slides were dyed with brilliant-cresyl-blue. A small drop of blood from the ear vein of the rabbit was placed on a dry slide and a cover glass put on. The count was performed after 10—20 minutes. For the reticulocyte percentage 1,000 red cells were counted.

In order to test the presence of an erythropoietic substance in the blood, 3 cc. of plasma (or serum) was injected intraperitoneally into normal or immunized rabbits, whose red blood count had been found stable during the previous few days. Haemolysis was carefully avoided and the visibly haemolysed samples were discarded. The haemoglobin and red cells of the recipient rabbits were observed on 5—6 successive days following injection. In some cases the reticulocytes were enumerated before the injection and also on the first 2—3 days after the injection.

The blood samples to be tested regarding erythropoietic substances were taken from anoxic patients with cardiac failure and from rabbits, after four hours' exposure to low athmospheric pressure. Blood from

Table 1.

No.	Age	Diagnosis	Duration of congestive symptoms	Oedema	Ascites	Liver enlargment	Dyspnea	Cyanosis	Hb % (Sahli)
1	48	Myodegeneratio et insufficientia cordis	1 year	—	—	+	+	+	80
2	51	Hypertonia. Myodegeneratio cordis. Bronchitis ac.	1 »	—	—	—	+	+	76
3	60	Myodegeneratio et insufficientia cordis	2 years	+	+	—	—	—	81
4	57	Hypertonia. Vitium valv. mitralis cordis. Insuff. cordis. Infarctus pulmon?	2—3 years	+	+	+	+	+	57
5	70	Myodegeneratio et insufficientia cordis. Bronchitis ac. Emphysema pulmonum	2 weeks	—	—	—	+	+	78
6	67	Myodegeneratio et insufficientia cordis	1 month	+	—	+	+	—	
7	70	Hypertonia. Myodegeneratio et insufficientia cordis. Arteriosclerosis	1 year	+	—	+	—	+	94
8	63	Myodegeneratio et insufficientia cordis	3 months	+	+	+	+	+	97
9	73	» » »	6 »	+	+	+	—	+	
10	57	» » » Fibrillatio atriorum. Anaemia secundaria	1 year	+	—	—	+	+	70
11	43	Insuff. et stenosis mitralis. Insufficientia cordis. Hypertonia	6 months	+	—	—	—	+	86
12	50	Asthma bronchiale. Laryngitis chron.	2 years	—	—	—	+	—	105
13	51	Hypertonia. Myodegeneratio et insufficientia cordis. Fibrillatio atriorum paroxysmalis. Lues III medicata	2 weeks	+	—	+	+	+	85
14	55	Hypertonia. Myodegeneratio cordis. Emphysema pulmonum. Bronchitis chronica	Several years	—	—	+	+	—	93
15	27	Vitium mitrale cordis. Insufficientia cordis. Bronchitis acuta	1 month	—	—	—	+	+	

patients with infectious diseases and from healthy persons was used for controls; for rabbit controls blood samples from normal rabbits were taken.

The human blood samples were drawn from the cubital vein into a 20 cc. syringe containing 4 cc. isotonic (3.8 %) sodium citrate; in a few of the first experiments no anticoagulants were used. The blood was then centrifuged within an hour and 3 cc. of the plasma (serum) was injected intraperitoneally into a) normal rabbits, b) into rabbits immunized against human plasma. The immunization against plasma of man was carried out by injecting 0.6—2.0 cc. citrated plasma from healthy persons into the ear vein of a normal rabbit, 2—4 times, at intervals of 3—6 days. After the immunization the red blood picture was observed until found stable. As whole blood was never injected, the blood group of the donor was considered to be of no importance. The group of *anoxic patients* consisted of 14 cases with congestive heart failure and a case with respiratory insufficiency (bronchial asthma) without signs of cardiac decompensation. Age, clinical diagnosis, duration of the case history, symptoms of congestion and blood status (Hb %) of the patients are shown in Table 1. Most of the patients had a comparatively short case history previous to admission into hospital. Four patients had taken digitalis.

Table 2.

Patients suffering from infectious diseases.

No	Age	Diagnosis	Duration of case history	Sedimentation rate	Hb % (Sahli)
1	40	Salpingo-oophoritis	10 days	37 mm/hour	77
2	44	Spondylarthritis chr. Hypernephroma	2—3 years	98 »	70
3	39	Pyosalpinx l. a.	2 months	103 »	73
4	46	Appendicitis ac. perfor. Peritonitis diffusa	2 weeks	50 »	68
5	30	Pyosalpinx l. sin. Salpingo-oophoritis	3.5 weeks	102 »	91

The control group consisted of patients suffering from some infectious disease in an acute stage (Table 2). Respiratory infections, hepatic disorders, and cases with circulatory failure were excluded.

The *donor rabbits* were exposed to lowered athmospheric pressure of 300—350 mm. Hg for 4 hours, corresponding to a simulated altitude of 6.5—7.5 km. As a rule the pressure was kept as low as the animal tolerated and varied slightly according to the differences in the critical level in the different animals. Blood samples were obtained by heart puncture performed within 5—10 minutes after the removal of the animal from the low pressure chamber. The citrated blood was centrifuged immediately and 3 cc. of the plasma was injected without delay into normal recipient rabbits.

For checking the erythropoietic effect of the low pressure used in

the main experiments, normal rabbits were subjected to the same pressure of the same duration.

The whole material was statistically treated. For calculation of the mean error of a single determination (m) and of the mean error of the mean (ε) FECHNERS equations $m = \frac{1.25 \cdot \Sigma\gamma}{n-0.2}$ and $\varepsilon = \frac{1.25 \cdot \Sigma\gamma}{(n-0.2)\sqrt{n}}$ were used, n being the number of the determinations and γ the deviation of the single determination from the mean. In a number of experiments m and ε were calculated as well according to the equations commonly used $m = \sqrt{\frac{\Sigma\gamma^2}{n-1}}$; $\varepsilon = \sqrt{\frac{\Sigma\gamma^2}{(n-1)\ n}}$. It was stated that both methods gave consistent values. In each group of experiments the single deviations from the mean followed the normal distribution curve of GAUSS.

Results.

Erythropoietic substances in the plasma of patients with stagnant anoxia.

Individual differences were stated in the red blood picture of the test animals. The erythrocyte count varied from 3.8 mill. to 6.0 mill., the haemoglobin percentage from 54 to 81, the colour index from 0.58 to 0.84. These fairly low values were most probably due to the fact, that the animals' cages were too small for sufficient exercise or to the temperature being too high, because, in later experiments, when the animals were kept in larger cages, at a low temperature, erythrocyte counts below 5 mill. were not stated. In a single rabbit the red blood picture is not quite stable, the daily variations of erythrocytes amounting to maximum 0.5 mill. and those of the haemoglobin to maximum 5 per cent. As the daily fluctuations in the red blood picture are considerable, the value of a single experiment is limited. It is essential, therefore, that the experimental as well as the control material should be large enough to allow tests of probability to be applied.

Control experiments.

In order to prove whether an injection of a foreign (human) protein might produce erythrocytosis in normal rabbits plasma obtained from healthy persons was injected into a control group of rabbits previously immunized against human plasma. In most cases the injection caused a decrease in the red cell count and the haemoglobin percentage which, however, rarely exceeded the

daily variations in the normal blood picture. In some rabbits there was a slight rise in the red cell count and Hb-percentage; the normal variations were never exceeded. No effect on the colour index was stated. The reticulocyte percentage after the injection was unaffected. In a few experiments on rabbits not immunized against human plasma the effect of an injection of human plasma was similar to that obtained in the immunized rabbits: a slight decrease in the erythrocyte count.

The red cell count, the colour index, and the haemoglobin percentage in the control experiments are given in Table 4. In the 4th column of the table the mean initial values are shown and the following columns, 5 to 10, give the mean deviations from the initial value on 6 days succeeding the injection. As may be seen, there is a slight tendency towards a decrease of erythrocytes and haemoglobin on all 6 days. Consequently, there cannot be any question of an erythropoietic activity of foreign proteins; on the contrary, their effect on the blood values is depressing rather than increasing. As desensibilization by immunization does not eliminate the depressing effect, it is probably not produced by the foreign proteins. The only agent that might be responsible for the decrease in the erythrocyte count is thus evidently the sodium citrate contained in the injected sample. In order to settle this question 0.6 cc. of a 3.8 per cent sodium citrate diluted in 3 cc. isotonic sodium chloride was injected intraperitoneally into 3 rabbits. As expected a slight decrease in the red cell count (200—300,000 cells/c.mm.) and in the haemoglobin followed (Table 4).

A second set of control experiments was considered necessary. In the main experiments, described below, blood was taken from patients suffering from congestive heart failure which is known to develop frequently on the basis of an infectious disease. For that reason it was considered essential to ascertain that the possible presence of erythropoietic substances was actually due to anoxia and not to products of tissue destruction in infections. Accordingly, blood plasma obtained from patients suffering from different infectious diseases without circulatory or respiratory complications was injected into a control group of 8 immunized rabbits. After the injection there was no increase or decrease in the red blood picture exceeding the daily variation in any of the rabbits. The mean deviations on 6 successive days following the injection are seen in Table 4 (third horizontal chief column).

It may be concluded that an infectious disease will not, as such, give rise to formation of any erythropoietins.

Experiments with plasma obtained from anoxic patients.

To test the presence of erythropoietins in the blood of an organism with stagnant anoxia, citrated plasma from patients with congestive heart failure (termed "stagnation plasma") was injected into 31 rabbits immunized against human plasma. The diagnosis, the different symptoms of congestion, and the duration of the case history are given in Table 1. There are some variations in the effect of the injection upon the red blood picture. The erythrocytosis promoting factors may be lacking in some patients (no reaction in *any* of the injected rabbits) or, some of the injected rabbits may fail to react in spite of there being some amount of erythropoietic substance in the injected plasma (reaction in *some* of the injected rabbits) (see Table 5). The material seems to split up into two groups. In the one group of experiments the changes in the red cell count and in the percentage of haemoglobin after the injection remains within the limits of normal daily variations, while in the second group the injection is followed by a definite increase in red cell count, maximum 30 per cent of the initial value = 1.4 mill/c.mm. and haemoglobin (max. 15 per cent), which cannot be considered normal variations. The erythrocytes and haemoglobin values are usually levelled again in 5 to 6 days. The increase is as a rule stronger in the

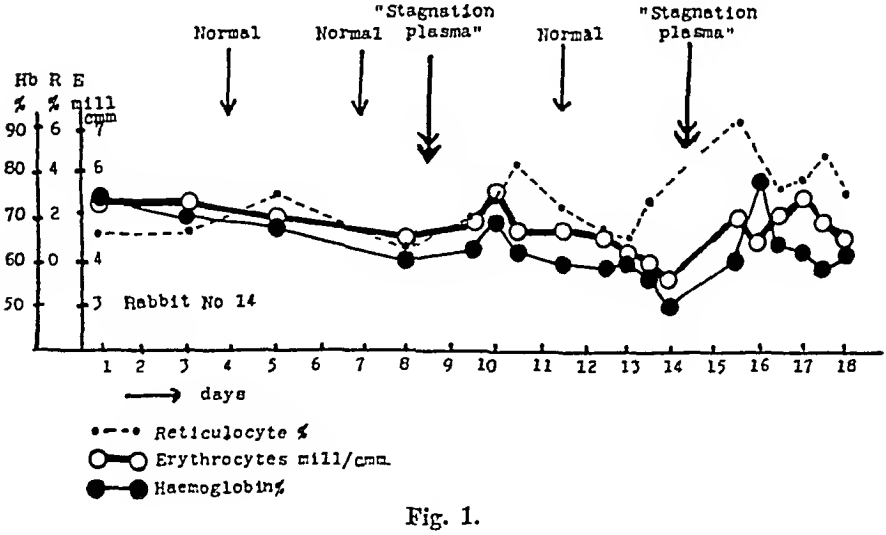

Fig. 1.

Difference mill/c.mm.

No. of recipient	Date	Initial value mill/c.mm	1st day	2nd day	3rd day	4th day	5th day	6th day
14	9. 3. 1945	4.9		+ 0.4	+ 1.1	+ 0.2		+ 0.2
1	17. 3. »	4.5		+ 1.1	+ 0.2	+ 0.4	+ 0.2	— 0.1
14	21. 3. »	3.8		+ 1.2	+ 0.7	+ 1.0		
1	4. 4. »	5.5		— 0.3	— 0.4	— 0.6	— 0.5	
6	7. 4. »	5.0		— 0.1	+ 0.1	+ 0.2	0.0	
14	11. 4. »	5.1		— 0.2	+ 0.3	+ 0.1	— 0.3	
16	16. 4. »	5.0	+ 0.2	+ 0.1	— 0.3	— 0.2	— 0.1	
18	— » —	4.5	+ 1.4	+ 1.0	+ 1.0	+ 0.2	+ 0.3	
14	21. 4. »	4.9	+ 0.4	+ 0.3	+ 0.7	+ 0.3		
17	— » —	4.4	+ 1.1	0.0	+ 0.5	0.0	+ 0.9	+ 0.3
1	26. 4. »	5.0	+ 0.4	— 0.4	— 0.7	— 0.4	— 1.0	
16	7. 5. »	4.9	— 0.1	0.0		— 0.5	— 0.7	
19	— » —	4.7	+ 0.5	+ 0.3		+ 0.2	— 0.2	
17	11. 5. »	4.8	+ 0.3	0.0	+ 0.3	+ 0.5	+ 0.4	
20	22. 5. »	5.2	+ 0.2	+ 0.9	— 0.1	0.0	— 0.5	
6	15. 6. »	4.7	— 0.3	— 0.2	— 0.4	— 0.2		— 0.6
18	— » —	4.6	+ 0.1	+ 1.4	— 0.1	+ 0.5	+ 0.4	— 0.1
18	23. 7. »	4.5	+ 0.1	+ 0.1	+ 0.4	+ 0.4		0.0
20	— » —	4.8	+ 0.4	+ 0.5	+ 0.4	+ 0.2		— 0.2
27	8. 10. »	4.4	+ 0.2	0.0	— 0.2	+ 0.5	+ 0.5	
28	— » —	4.6	— 0.3	— 0.6	+ 0.1	— 0.6	— 0.8	
27	19. 10. »	4.9	— 0.2	— 0.1	— 0.4	— 0.4		— 0.3
28	— » —	4.4	+ 0.4	+ 0.3	+ 0.5	— 0.3		+ 0.1
30	18. 2. 1946	4.4	— 0.4	+ 0.6	— 0.5	+ 0.1	— 0.4	
37	— » —	3.9	+ 0.5	+ 0.2	— 0.5	— 0.3		
32	23. 2. »	5.1	0.0	— 0.6	— 0.8	— 0.2	— 0.1	
39	— » —	4.4	+ 1.0	+ 0.1	+ 0.3	— 0.2	— 0.2	
40	— » —	4.5	— 0.4	— 0.1	— 0.4	— 0.2	— 0.3	
45	12. 3. »	5.4	+ 0.4	+ 0.1	— 0.1	— 0.3		— 0.4
46	— » —	4.7	+ 0.1	— 0.4	— 0.2	— 0.5	— 0.5	— 0.5
50	— » —	4.6	+ 0.3	— 0.8	+ 0.6	+ 0.5		+ 0.3
	Mean	4.7 ± 0.06	+ 0.25 ± 0.08	+ 0.22 ± 0.08	+ 0.07 ± 0.10	+ 0.01 ± 0.07	— 0.15 ± 0.10	—0.11 ± 0.11
	n	31	25	31	29	31	20	12

Table

1	2	3	4	5
Donors	Recipients		Initial value	1st day
Normal persons	Immunized rabbits	Er mill/c.mm Hb % Index n	4.7 ±0.13 64 ±2.1 0.69±0.01 14	0.00 ±0.06 —0.2 ±0.8 —0.018±0.008 14
Normal persons	Non immunized rabbits	Er mill/c.mm n	4.8 ±0.09 4	0.00 ±0.05 3
Patients with infectious diseases	Immunized rabbits	Er mill/c.mm Hb % Index n	5.0 ±0.11 68 ±2.2 0.69±0.020 8	+0.02 ±0.12 +0.3 ±1.3 +0.010±0.013 8
Patients with congestive heart failure	Immunized rabbits	Er mill/c.mm Hb % Index n	4.7 ±0.06 67 ±1.3 0.74±0.012 31	+0.25 ±0.08 +1.8 ±0.9 —0.018±0.009 25
Patients with congestive heart failure	Non immunized rabbits	Er mill/c.mm n	4.8 ±0.09 7	—0.40 ±0.24 3
Normal rabbits	Normal rabbits	Er mill/c.mm n	5.7 ±0.25 5	—0.02 ±0.03 5
Anoxic rabbits (low pressure)	Normal rabbits	Er mill/c.mm Hb % Index n	5.1 ±0.48 71 ±1.0 0.70±0.010 38	+0.16 ±0.06 —0.3 ±0.4 —0.020±0.008 36
Sodium citrate in 3.8 % solution	Normal rabbits	Er mill/c.mm n.	5.0 ±0.10 3	—0.27 ±0.02 3

red cells than in the haemoglobin, in consequence, the colour index shows a slight tendency to decrease after the injection. In the test animals which reacted to the injection with an erythrocytosis a reticulocyte percentage above the normal often is stated on the first few days after the injection. Fig. 1 shows a typical reaction with increased values for erythrocytes, haemoglobin, and a reticulocytosis.

As mentioned before, single experiments are generally of limited value. The data should be treated as a whole, and the experiments with "stagnation plasma" compared with the corresponding controls. Tables 3 and 4 give the red cell count,

4.

6	7	8	9	10
Difference on				
2nd day	3rd day	4th day	5th day	6th day
—0.09 ±0.07 —2.1 ±1.0 —0.013±0.006 14	—0.08 ±0.13 —0.9 ±0.7 —0.003±0.015 13	—0.16 ±0.11 —1.9 ±1.0 +0.001±0.016 13	—0.32 ±0.16 —2.0 ±0.7 +0.026±0.014 9	—0.13 ±0.14 —2.8 ±0.7 —0.010±0.016 6
—0.21 ±0.05 4	—0.23 ±0.12 4	+0.15 ±0.13 2	—0.2 1	+0.20 ±0.09 2
—0.15 ±0.14 —0.6 ±0.7 +0.007±0.021 7—8	—0.15 ±0.07 —1.9 ±1.0 +0.004±0.016 8	—0.08 ±0.07 —1.3 ±1.6 +0.014±0.017 6—8	—0.30 ±0.14 —4.1 ±1.2 0.000±0.019 7	
+0.22 ±0.08 +0.4 ±0.9 —0.027±0.010 30—31	+0.07 ±0.10 +0.4 ±1.3 —0.018±0.009 21—29	+0.01 ±0.07 +0.4 ±0.9 —0.009±0.009 31	—0.15 ±0.10 —2.4 ±1.3 —0.023±0.011 18—20	—0.11 ±0.11 —1.8 ±0.9 —0.008±0.027 12—13
—0.33 ±0.22 7	—0.06 ±0.19 7	—0.23 ±0.10 6	+0.05 ±0.13 6	+0.05 ±0.25 4
—0.16 ±0.07 5	—0.18 ±0.07 5	—0.18 ±0.07 5	—0.08 ±0.07 5	—0.02 ±0.08 5
+0.06 ±0.06 +0.4 ±0.4 —0.011±0.007 37	+0.19 ±0.07 +0.5 ±0.6 —0.013±0.009 34	+0.10 ±0.07 +1.2 ±0.6 —0.006±0.008 34	+0.01 ±0.06 —1.0 ±0.7 —0.010±0.008 27	—0.02 ±0.08 —0.7 ±0.6 +0.003±0.010 17
—0.13 ±0.07 3	—0.27 ±0.07 3	—0.15 ±0.04 2	—0.1 1	

haemoglobin, and colour indexes in experiments with "stagnation plasma". Table 3 gives the individual erythrocyte values, whereas the haemoglobin, and the index values are treated and represented in the same way as the control data in Table 4 (4th chief horizontal column).

The increase in the red cells, as seen from Table 4, is statistically significant on the 1st and 2nd day after the injection, whereas there is no significant difference (increase or decrease as compared with the initial value) on the 3rd to 6th day. In the haemoglobin a corresponding mean increase takes place on the first few days; this is, however, statistically, only probable. In accordance with

the fact that the number of red cells is more increased than the haemoglobin, the colour index shows a tendency to decrease on the 1st and 2nd days after the injection in the cases that have reacted. The mean values are of very little significance as the colour index does not represent an independent variable.

It must be borne in mind that an injection of citrated plasma tends to lower the erythrocyte count. On that account the results obtained with injections of "stagnation plasma" appear more favourable. Obviously the comparison must be made between the values in the control group and the values in the main experiments and *not solely between the initial value and the values on the days succeeding the injection.* Such a comparison is illustrated in Fig. 2. The upper curve in Fig. 2 gives the mean deviations from the initial value in the erythrocyte count on the 1st—6th day following the injection of plasma from patients with stagnant anoxia, the lower curve the corresponding mean deviations following injections of normal human plasma. The curves differ from each other in points corresponding to the 1st and the 2nd day for 300,000 cells, the mean errors varying between about 60,000 and 80,000 cells (marked with circles). This difference is statistically significant. On the other hand the difference gradually declines, being on the 3rd—6th day without significance, as might be expected following one single injection.

It is thus evident, that an injection of plasma from patients suffering from tissue anoxia promotes erythrocytosis in rabbits previously immunized against human plasma. Rabbits, which were not immunized with human plasma and which served as test animals in a number of experiments in the beginning of this study did not react, however, on injections of stagnation plasma and were, therefore, considered unsuitable as test objects. In this group of rabbits no increase in the erythrocyte count or haemoglobin was encountered, although the same plasma was effective when injected into immunized rabbits (Table 4 5th horizontal chief column). The question naturally arises whether the obtained reactions are due to the immunization sensitizing the animals against the erythrocytosis promoting factor, or, whether the immunization only neutralizes the possibly deleterious effect of foreign protein injection allowing, on that account, a favourable reaction to appear. In order to settle this question it seemed pertinent to carry out experiments with homologous plasma injections.

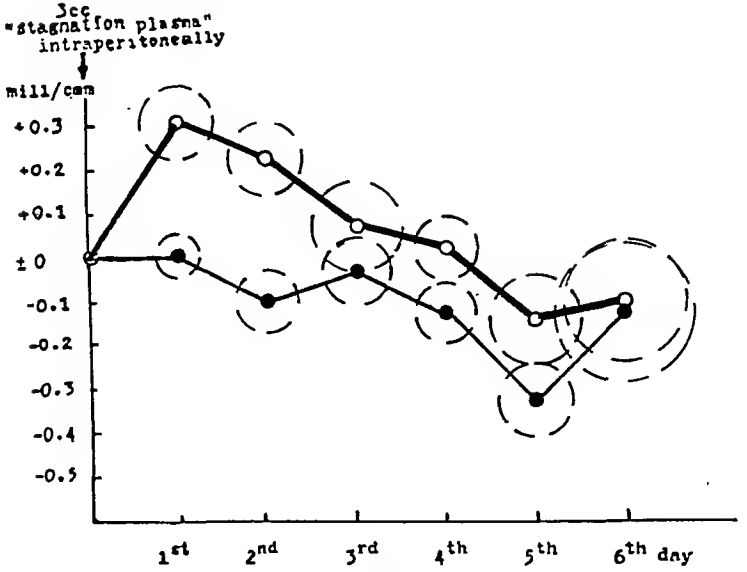

Fig. 2.

The Formation of Erythropoietic Substances in Acute Low Pressure Anoxia.

Control experiments.

For the purpose of testing the influence of intraperitoneal injections of plasma, obtained from animals of the same species, upon the red blood picture, citrated plasma from untreated rabbits was injected intraperitoneally onto 5 normal rabbits. In each of the latter the injection was followed by a very slight decrease in the red cell count, never exceeding the range of normal variations, whereas the haemoglobin showed no decrease. The colour indexes were consequently slightly increased. It may be concluded that plasma obtained from normal rabbits has *no erythropoietic effect* when injected intraperitoneally into other normal rabbits.

Experiments with plasma obtained from rabbits exposed to low pressure.

Thirty-eight normal untreated rabbits were given injections of plasma taken from rabbits which had been kept for four hours under a barely tolerable low pressure. The injections of the plasma obtained after the exposure (termed anoxic plasma) were followed,

in most cases, by an increase in the red blood count. The increase, however, seldom exceeded the variations which may occur spontaneously. In some cases increased haemoglobin percentages were noted as well. Fig. 3 shows a typical response without any change in the Hb-percentage. In Table 4 the deviations from the initial value of the erythrocyte count after the injections are given (7th horizontal column). It appears that an increase on the 1st and 3rd days after the injection borders on significance. The deviations on other days may be regarded as random variations. Similarly as in the experiments with human plasma the results must, however, be chiefly evaluated on the basis of a comparison between values recorded in the control experiments and those obtained in the main experiments with injections of "anoxic plasma" and not by comparison of the initial value with the post-injection values alone. In Fig. 4 the mean deviations in the erythrocyte count in both experimental groups are plotted against time in days after the injection. The mean errors are marked with circles. As may be seen, the curve representing the values after injections of anoxic plasma runs definitely apart and higher than the curve representing the control experiments. The difference between the two curves is highly significant in points corresponding to the 1st, 3rd and 4th day, on other days the difference is, however, not significant. It may thus be concluded that the plasma from animals rendered anoxic by exposure to lowered athmospheric pressure, has an erythropoietic effect when injected intraperitoneally. This effect is strongest on the 3rd and 4th days, *i. e.* somewhat delayed, when compared with the effect of injections of human "stagnation plasma" into immunized rabbits. This shows that the result obtained in the experiments *with human plasma are not caused by the immunization, as similar results may be obtained with injections of homologous anoxic plasma in non-immunized animals.* The distribution of reactions in the different donors and recipients is shown in Table 5. An increase from the initial value of, or above 0.5 mill.E/c.mm. on one of the first 4 days is marked with +, an increase from

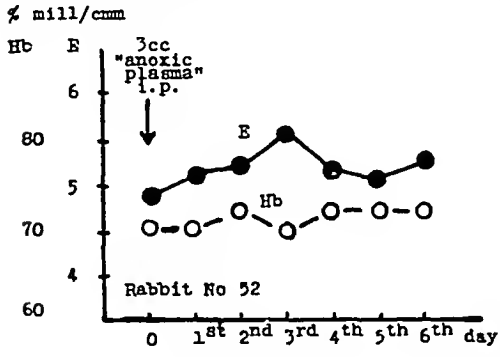

Fig. 3.

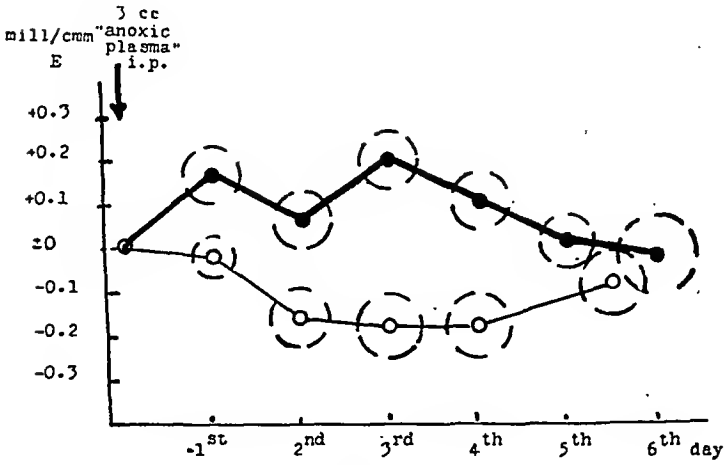

Fig. 4.

0.1—0.4 mill.E/c.mm. with ±, and cases which show no increase in the red cell count on the 4 first days are marked with —.

As the erythrocytosis promoting effect of low pressure is a fact proved by several authors a closer study of the changes in the red blood picture of the exposed rabbits was considered superfluous. It was however essential to know that the pressure and the duration of the exposure used was sufficient to produce erythrocytosis in rabbits. For this purpose 2 rabbits were ex-

Table 5.

No. of patient	No. and reaction of recipient rabbits	No. of donor rabbits	No. and reaction of recipient rabbits
1	14: +	32	22: + 29: ± 37: ±
2	1: + 14: +	16	18: — 28: + 39: ±
3	1: — 6: ± 16: ± 18: +	39	51: — 52: + 53: +
4	14: ±	a	54: ± 55: ±
5	1: ± 14: + 17: + 17: +	b	56: + 57: ± 58: ±
6	16: — 19: +	54	59: — 60: — 61: —
7	20: +	56	63: ± 64: +
8	10: ±	59	72: — 73: ± 74: ±
9	6: — 18: +	87	88: + 89: ±
10	18: ± 20: +	95	90: + 91: — 92: ±
11	27: + 28: ±	112	90: + 100: ± 102: + 104: + 105: +
12	45: ± 46: ± 50: +	115	106: + 107: +
13	27: — 28: +	101	120: ± 121: +
14	30: + 37: +	?	110: ± 117: +
15	32: — 39: + 40: —		

\+ = increase in E. count as compared with the initial value on some of the four first days after injection ≧ + 0.5 mill. E/cmm.
± = increase 0.1—0.4 mill. E/cmm.
— = no increase on any of the four first days after injection.

posed to the usual pressure of 300 to 350 mm. Hg for four hours and their blood picture observed in the same way as that of the recipient rabbits. In both rabbits the exposure gave rise to erythrocytosis, well beyond the normal variations, viz. + 0.7 and + 1.0 mill/c.mm. respectively on the third day. It is evident that the exposure in question is sufficient to promote erythrocytosis.

Discussion.

Anoxic erythrocytosis is generally described to be due to an increased production of red cells, directly caused by the lowered O_2 tension in the bone marrow. On the other hand, attempts to show an increased metabolism in bone marrow *in vitro* as a response to lowered O_2 tension have failed, whereas the bone marrow shows in vitro a definite increase in the oxydative metabolism when the animals have previously been exposed to low athmospheric pressure (WARREN, 1941). This is interpreted as showing that the low local oxygen tension in the bone marrow cannot, as such, be the stimulus for increased blood formation. The results of the present work point in the same direction. The possibility to induce an erythrocytosis in a normal organism by injections of plasma from an animal suffering from anoxia seems to be well established. The bone marrow of the normal recipient is not, naturally, in want of oxygen and, accordingly, the erythrocytosis is brought about by some humoral factor which might probably be the physiological erythropoietic stimulus also in the anoxic animal: a true erythropoietin. On that account it might be expected that the donor organism, whose plasma has been proved effective in promoting erythrocytosis, would show polycythemia. In donor rabbits this cannot be tested as the amount of blood removed for plasma injections is so great that the rabbit becomes anaemic. In patients with congestive failure, on the other hand, the blood values are usually low or normal, even in cases in which erythropoietically active substances are demonstrable in the plasma. An explanation of this fact may be found in assuming that the reacting mechanism is deficient or that an increased destruction of red cells counterbalances the red cell production. The latter assumption is supported by EHRSTRÖM who stated an increased reticulocytosis, and urobilin excretion in patients with cardiac failure regardless of their blood values.

The mechanism of the erythrocytosis manifested in peripheral blood counts in response to the action of the erythropoietins requires further study. Yet, the most plausible assumption seems to be that the production of red cells is increased. The occurrence of reticulocyte responses strongly favours this view. It is hardly probable that the erythrocytosis is apparent only and caused by haemoconcentration. Even if assumed that the injection causes some haemoconcentration, either by a general loss of fluid from the organism, or by exudation of fluid into the peritoneal cavity, there would be some difficulty in explaining, why the injection of normal plasma has no such effect. Moreover, if the erythrocytosis were due to haemoconcentration no change in the colour index would be expected. In fact, the colour index is decreased in most of the cases reacting to the injection. Due to following reasons the erythrocytosis cannot either be caused by the emptying of blood depots. The erythropoietins are easily destroyed in vitro (as shown by experiments to be published at a later date) and it is most probable that the inactivation occurs in vivo quite as rapidly. The erythrocytosis appears, however, in a number of recipient rabbits only on the 2nd or 3rd day after the injection. As the emptying of blood depots is known to be a momentary reaction, it is not conceivable, why the organism lags reacting for three days.

The erythrocytosis setting in as early as on the 1st and 2nd day after the injection is an incident of interest. In cases of pernicious anaemia a reticulocytosis is usually not observed until the 3rd or 4th day after liver administration. The case is similar in other macrocytic anaemias treated with pteroylglutamic acid (WILTER, SPIES and KOCH, 1945). The results obtained in the present paper are, however, in good accordance with experiments performed with *radioactive iron* (WHIPPLE et al.). In these experiments the ingested iron was detected in some red cells already in 2 hours, the interpretation of which may be that new erythrocytes are formed within a few hours. KARVONEN, when considering the quantitative facts known about the erythron, arrives at similar estimates.

The rather rapid disappearance of the induced erythrocytosis should be discussed. As a rule the duration of the erythrocytosis was a few days only, as mentioned before. Bearing in mind, that the normal recipient is balanced as regards the oxygen transmitting elements at the moment of injection, it seems only nat-

ural that it strives to reestablish the balance disturbed by the extra output of red cells. This might be effected by means of a reduction in the formation of red cells, or, less probably so, by an increased destruction as well. If the span of life of a rabbit erythrocyte which is not exactly known, were, say 25 days, the blood volume remaining stable, about 300,000 new cells per c.mm. would be formed each day. Thus, by reducing the red cell production the blood values would easily be levelled in a few days.

The objection may be made that the induced erythrocytosis is rather slight on an average. Yet, a very great response could hardly be expected as the amount (3 cc.) of the injected plasma is small, representing only about 1/20—1/30 of the total plasma of a rabbit. The injected amount was not increased in view of the fact that as little as 3 cc. anoxic plasma could produce an effect of the same order as obtained in normal rabbits by exposure to low pressure. Furthermore, an intraperitoneal injection of larger amounts of plasma might have affected the fluid balance in quite an unexpected way.

Moreover, it should be noted that the apparent slightness of the average increase in the erythrocyte count after the injections was mainly due to the great number of cases failing to react and not to the slightness of the response when present.[1]

Summarizing it may be said that formation of erythropoietic substances under anoxia seems to take place. These erythropoietins appear both in the plasma of organisms with *acute* arterial anoxia, as well as in *chronic* stagnant anoxia, *i. e.* in patients with congestive heart failure. These substances are effective both in normal and in anaemic animals and may represent the physiological stimulus of blood formation.

Summary.

Experiments on the existence of erythropoietic substances in the plasma of chronically as well as acutely anoxic organisms were carried out. As test animals normal rabbits were used.

Plasma from 15 patients with chronic circulatory or respiratory (1) insufficiency was injected intraperitoneally into 31 normal rabbits immunized against human plasma and into 7 untreated

[1] The reactivity of the rabbits seems to vary according to the general condition of the animal. *E. g.* in summer, when the room temperature was comparatively high, practically no reactions were obtained.

rabbits. In the immunized group the injection caused on the 1st and 2nd day a statistically significant although slight average increase in the red cell count. Reticulocyte responses were obtained as well. The untreated rabbits failed to react to the injection.

As control plasma from normal persons and from patients with infectious processes was injected into 22 immunized and 4 untreated rabbits. In these control groups the injection called forth no increase in the red cells; on the contrary there was a slight decrease.

Plasma drawn from normal rabbits within 5—10 minutes after an exposure to lowered athmospheric pressure (300—350 mm. Hg) for 4 hours was injected into 38 normal rabbits. The recipients showed slight average increase in the erythrocytes statistically significant on the 1st, 3rd and 4th day following the injection. Plasma from normal not anoxic rabbits had no erythropoietic but a slight depressing effect.

In order to avoid any amount of haemolysis citrated plasma was used in all experiments. It is therefore improbable that products of red cell destruction in vitro would be responsible for the erythropoietic effect. The depression in the red cell count following injection of normal plasma, was shown to be due to the sodium citrate as a corresponding decrease was obtained in control experiments with i. p. injections of sodium citrate diluted with isotonic normal saline.

We are greatly indebted to professor P. Soisalo, M. D., Chief of the Kivelä Hospital, professor Ö. Holsti, M. D., Chief of the University IIIrd Medical Department and professor Fr. Saltzman, M. D., Chief of the Maria Hospital Medical Department, for their kind permission to have blood samples taken from patients warded at their Hospitals and it gives us great pleasure to express our gratitude to them.

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On the Determination of the Phosphatide-Content of Serum.

By

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The method most frequently used for the quantitative determination of phosphatides in blood and tissues is based on the following principle (HOPPE-SEYLER): Extraction of total lipids, removal of non-lipid phosphorus components, and determination of phosphorus after ashing of the purified lipid extract, the figure for phosphatide being obtained by multiplication by 25.

Despite its simplicity and various improvements in the course of time the method cannot be described as wholly satisfactory, and the initial phases of the method will be dealt with in detail below.

The Primary Lipid-extraction.

Lipids may be extracted from blood and tissues in two ways: The substrate may be extracted *after drying* by prolonged treatment with comparatively small quantities of fat solvents, or — as introduced by BLOOR in 1914 — the material, especially blood, may be extracted *moist* by being treated for a short period with comparatively large quantities of the solvent.

When extracting according to the former method the chief difficulty is to obtain a sufficiently careful and yet effective drying of the tissues. If this is achieved by simple drying in open air a partial oxidation of the non-saturated fatty acids of the

lipids — especially the phosphatides — takes place, whereby substances are formed which are insoluble in petroleum ether, insoluble or difficult to dissolve in ether, and easily dissolved only in alcohol. The material should therefore be dried as speedily as possible, at the lowest possible temperature, and with the least possible exposure to the oxygen of the air.

BLOOR (1914, 1915) simply lets a fine jet of serum pass into a mixture of alcohol and ether (3 + 1) which is then brought to the boil and left to boil for a few seconds. This simple method of extraction, which has become very popular, has later been improved in various ways. MAN and GILDEA (1932—33) found that when plasma is boiled for 1 hour with alcohol-ether 5—31 % more fatty acids are obtained than when the BLOOR method of extraction is applied, whereas longer boiling does not materially increase the yield. BOYD (1936) has most convincingly explained the importance of the correct quantitative proportion between extraction medium and substrate and demonstrated that complete extraction cannot be obtained with less than 20 volumes of solvent for serum and plasma respectively, and 30 volumes for total blood. If such sufficient dilutions are used, the yields is not increased whether boiled for 5 minutes or 2 hours, and serum-lipids are dissolved easily and quickly even in cold alcohol-ether. MAN and GILDEA, however, use a smaller volume of solvent for which they must compensate by heating for a longer period.

Author's Own Investigations.

1) In order to evaluate the efficiency of BLOOR's extraction method in comparison with the more elaborate methods applied to dried substance we used the following three principles of extraction for the same serum:

I. 5 ml serum were dried quickly in a flat glass dish by means of a current of hot air, and the drying was completed in the exsiccator over $CaCl_2$ in vacuo, powdered in a mortar by means of fine sea sand, and extracted in the *Soxhlet* apparatus with 40 ml of absolute alcohol for 9 hours. II. 10 ml serum were dried in the same manner and extracted in a modified *Soxhlet* apparatus described by THANNHAUSER and SETZ (1936), who also indicate the very careful method of drying. Extraction was carried out with methylalcohol-chloroform (70+30) for 10 hours. III. 2 ml serum were added drop by drop to 50 ml warm absolute alcohol which were then left to cool.

The total-lipid and phosphorus content of the filtrate of the three extractions are seen in Table 1.

Table 1.

Extraction method	Total-lipids mg%	Lipid-P mg%
I Dried serum (Soxhlet apparatus)	1355	7.69
II » » (a. m. Thannhauser and Setz)	1540	9.38
III Serum added to absolute alcohol	1600	10.27

It will be seen that *extraction of total-lipids and phosphatide by dripping serum into (25 vol.) absolute alcohol at least yields as much as a prolonged extraction of carefully dried, powdered serum.*

2) Several experiments have shown that there is no difference in the yield when extracting lipids and particularly phosphatides whether absolute alcohol, alcohol-ether (3 + 1), or alcohol-acetone (1 + 1) is used, and we are therefore able to establish that *absolute alcohol alone* is quite adequate and gives the same result as mixtures containing ether or acetone.

3) So far there has been some doubt whether extraction of short duration after the BLOOR method yields as much as boiling for 1 hour, as suggested by MAN and GILDEA, and, therefore, we have in the following experiments examined how the alcohol extraction depends on temperature and extraction time. In one series of experiments serum was added drop by drop to 20 vol. absolute alcohol of various temperatures (20° C.—80° C.) and then left to cool, and in another series it was boiled with absolute alcohol for increasingly long periods (15 min.—3 hours) (Table 2).

Table 2.

Temperature and extraction time	Total-lipid mg%	Lipid-P mg%
20° C. (added drop by drop)	460	4.17
40° C. » » » »	580	4.20
60° C. » » » »	560	4.10
80° C. » » » »	575	4.07
15 minutes' boiling	570	4.09
1 hour's »	575	4.13
3 hours' »	565	4.17

Extraction of serum with 20 vol. absolute alcohol.

From the above it will be seen that in the case of phosphatide extraction no increase of the yield could be observed at rising temperatures and longer time of extraction. Even by boiling for as much as 3 hours no more phosphatide was extracted than with

cold alcohol. This was also the case with the total-lipid except that in this experiment cold alcohol extracted less than warm alcohol, although no difference was observed in other experiments.

Purification of the Primary Lipid-Extract.

A quantitative determination of the phosphatides by a phosphorus determination of the evaporated, ashed lipid-extract depends not only on the total extraction of the phosphatides but also on the absence of inorganic phosphates or other phosphorous, non-lipid components in the extract. Is, then, a purification of the primary lipid-extract necessary before ashing and analysis for phosphorus?

Bloor (1914) found that inorganic phosphate is soluble in alcohol-ether in small quantities, but also observed that when blood was treated with alcohol-ether water-soluble phosphates could not be demonstrated in the extract. This was confirmed by Man and Peters (1933) and Wilson and Hansen (1935—36). Page, Kirk, Lewis, Thompson and van Slyke (1935) maintain, on the other hand, that by purification of the primary lipid-extract in petroleum ether it is possible to remove 20—30 % of the phosphorus soluble in alcohol-ether, for which reason they recommend to re-dissolve the evaporated lipid-extract in petroleum ether before the phosphorus determination. But Kirk, Page and van Slyke (1934) also point out that a re-extraction of lipids with petroleum ether is incomplete if the alcohol-ether extract is evaporated at a temperature above 60° C. and even more so at 75° C. Ellis and Maynard (1937) state that petroleumether only is a suitable solvent for phosphatides if the preceding evaporation of the primary extract takes place in vacuo. By evaporation of the primary extract in vacuo, N-atmosphere, and at a temperature below 30° C. Man (1937) was able to recover 95—100 % of the phosphorus soluble in alcohol-ether as lipid phosphorus soluble in petroleum ether, and this was confirmed by Williams, Erickson, Avrin, Bernstein and Macy (1938).

Own Investigations.

1) As some uncertainty prevails with regard to the nature of the primary lipid-extract and the advisability of re-dissolving

it in petroleumether, the influence of the various methods of evaporating the alcohol extracts on its solubility in petroleum ether has been investigated.

In the following experiment (Table 3) the serum lipids which had been extracted in absolute alcohol were evaporated partly in open air at different temperatures, partly in the most careful manner, that is in vacuo, under nitrogen, at a temperature of 37° C., and with exclusion of atmospheric oxygen until solution in petroleum ether has been completed. For the sake of comparison the phosphorus content in the directly ashed primary lipid-extract was determined.

Table 3.

Method of evaporation	P in mg%
Atmospheric air, 42° C. (3 3/4 hours)	12.6
» » 42° C. (18 hours)	12.4
» » 59° C. (2 1/2 hours)	12.6
» » 104° C. (30 minutes)	12.4
Vacuum, N-atmosphere, 32—38° C. (45 minutes)	12.5
(Primary lipid-extract, not re-extracted)	13.3

Evaporated lipid-alcohol extract re-extracted in petroleum ether.

It will be seen that the phosphorus content (after ashing) was at any rate smaller in the petroleum ether extract than in the primary alcohol extract which had not been re-extracted. Further, that even the most careful evaporation with exclusion of the atmospheric oxygen and at a low temperature did not yield more than when the alcohol extract was evaporated in open air at temperatures as high as 104° C. In any case petroleum ether only extracted about 94 % of the phosphorus content present in the alcohol extract. This may either be due to the fact that petroleum ether purifies the primary lipid-extract of non-lipid phosphorus components or that during the evaporation of the alcohol extract the phosphatides undergo certain changes which make them less soluble in petroleum ether. But the circumstance that the yield of phosphorus is unchanged whether evaporation takes place at temperatures above 100° C. and in open air or at 37° C. without atmospheric oxygen goes against the latter theory.

2) For a closer investigation of the matter we have in the next experiment (Table 4) re-extracted the alcohol extract, evaporated at 42° C., with different solvents and compared the yield of phosphorus with the content of the directly ashed, primary alcohol extract:

Table 4.

Extraction medium	P in mg%		
	I	II	III
Petroleum ether	9.9	12.5	
Benzene	10.3	13.0	
Carbon-tetra-chloride	10.45		
Chloroform	10.6	13.2	12.1
(Primary lipid-extract not re-extracted)	10.68	13.4	12.3

Re-extraction of the primary lipid-extract with different solvents.

The experiment shows that of the four lipid solvents used petroleum ether yields the smallest quantity of phosphorus, viz. 93 % only of the phosphorus present in the primary extract, while the solubility increases with benzene, carbon-tetra-chloride, and boiling chloroform, which dissolves 98.5—99 % of the phosphorus in the evaporated primary alcohol extract.

3) Attempts at extracting an evaporated phosphate solution (KH_2PO_4) with boiling chloroform showed that only 1.8 % of the original amount of phosphorus — that is, a minimum amount, within the limits of the experimental error — was recovered in the chloroform. Consequently, since chloroform does not dissolve inorganic phosphate in demonstrable quantities, and Table 4 shows that chloroform dissolves as much as 99 % of the phosphorus content of evaporated primary alcohol extract, it may be established that *when extracting serum lipids with absolute alcohol with simultaneous precipitation of proteins, inorganic phosphate does not pass into the filtrate* (paper filter). Furthermore, it may be concluded that the reason why the primary petroleum ether extract contains less phosphorus than the primary alcohol extract is not that the remaining lipids by treatment with petroleum ether have been purified of existing inorganic phosphates, but simply that *petroleum ether always is a less effective solvent of phosphatides.*

Discussion.

The present investigations show that with comparatively small quantities of serum it is quite safe to relinquish the old and more laborious methods for lipid extraction, which presuppose drying of the tissues and prolonged treatment with the solvent, in favour of the Bloor principle according to which liquid serum is brought into direct contact with the extraction medium and the extrac-

tion is speedily completed. It is, however, a condition (BOYD) that the ratio 20 parts solvent/1 part serum is maintained. As for extraction medium, we have been able to establish that absolute alcohol alone yields quite as much as mixtures of alcohol and ether or acetone. We have further demonstrated that absolute alcohol extracts serum-lipids instantaneously and that even without heating the extraction — as far as phosphatides are concerned — is quantitative, which agrees with BOYD's observations. Consequently, we have not been able to confirm MAN and GILDEA's investigations according to which the extraction of serum-lipids is not completed until after 1 hour's boiling.

Contrary to PAGE, KIRK, LEWIS, THOMPSON and VAN SLYKE we found that the alcohol extract of serum contains no phosphorous components except phosphatides, for which reason a purification of the lipid-extract must be considered superfluous. Furthermore, as we have demonstrated that petroleum ether is a rather inefficient solvent of phosphatides, it must be considered particularly unsuitable for the purpose of re-extraction. This confirms investigations made by KRAINICH (1938), ERICKSON, AVRIN, TEAGUE and WILLIAMS (1940) and ARTOM and FREEMAN (1940), who also found that both benzene and chloroform are superior to petroleum ether for the re-extraction of phosphatides.

Conclusion.

1) Serum should be extracted (lipids) in its moist, natural state and not after preceding drying.

2) Absolute alcohol extracts lipids and particularly phosphatides quantitatively and is just as efficient as mixtures with ether (or acetone), and temperature and extraction time are of minor importance when the ratio of 1 part serum to 20 parts solvent is maintained.

3) The phosphatide-content of serum may be determined by phosphorus determination of the directly ashed alcohol extract, and an additional purification of the lipid-remnant in the petroleum ether is both superfluous and inexpedient, as the filtrate after alcohol extraction (with simultaneous precipitation of proteins) does not contain inorganic phosphates, and because in all circumstances petroleum ether is a less efficient solvent of phosphatides and inferior to benzene, carbon-tetra-chloride and, particularly, to chloroform.

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On the Colorimetric Determination of Phosphorus with "Amidol".

By

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"Amidol" (Agfa) was introduced in 1935 by MÜLLER as a substitute for the less easily obtained Eikonogen as the reducing principle in colorimetric determination of phosphorus. When amidol (2,4-diamidophenolchlorhydrate) reduces phospho-molybdic acid, a blue compound is formed the colour intensity of which — determined *e. g.* in the photometer — is directly proportional to the phosphorus content within certain limits.

As this substance did not procure sufficiently exact results, some of the factors influencing the course of the colour development have been more closely investigated — such as the importance of the acidity, the age of the amidol powder, the freshness of the reagents prepared, and the temperature.

Amidol (Agfa) and ammonium-molybdate (Merck) were used in the same proportion as stated by MÜLLER (1935): 2 ml molybdate solution (1.25 g ammonium molybdate in 100 ml sulphuric acid water) and 2 ml amidol-sulphite solution (0.2 g amidol + 2 g acid sodium sulphite in 100 ml water) in a total volume of 8 ml (BRUN 1939).

1) An investigation of the colour development when the *normality* of the colour reaction mixture was varied gave the following colour curves (see figure).

None of the curves are perfect, but a normality of 0.66 n gives the most favourable colour curve with an almost maximum colour development during 10 minutes and then an increase of only 0.32 % in 10 minutes. The normality may at any rate be varied between

0.59 and 0.73 n without the curve changing, and at that normality the blank is stable for at least 24 hours while, if the solution is less acid, it turns bluish after a longer or shorter period of time.

2) According to BRUN (1939) quite fresh, that is white, *amidol powder* should be used, while older powder which has a more greyish appearance is unsuitable. The following experiments (Table 1) elucidate this problem: Amidol powder of three different qualities was tested in the photometer against the same phosphate solution.

Table 1.

Amidol powder	Extinction coefficient
greyish-white	0.636
grey	0.640
greyish-black	0.658

It will be seen that the difference of the colour intensity is slight (and slighter when the solutions are 10 and 17 days old). The older amidol powder shows a tendency to produce higher values but seems otherwise suitable for the purpose (obviously when using standard curves from the same powder).

3) An attempt to investigate the importance of the freshness of the *amidol solution* was made in the following experiments (Table 2):

Table 2.

Age of amidol solution	Extinction coefficient
1 day	0.597
7 days	0.600
17 »	0.602
22 »	0.614
28 »	0.618

Table 3 shows the importance of the freshness of the *molybdate solution* (1.24 n in sulphuric acid):

Table 3.

Age of solutions molybdate	amidol	Extinction coefficient
fresh	fresh	0.491
15 days	fresh	0.502
fresh	15 days	0.509

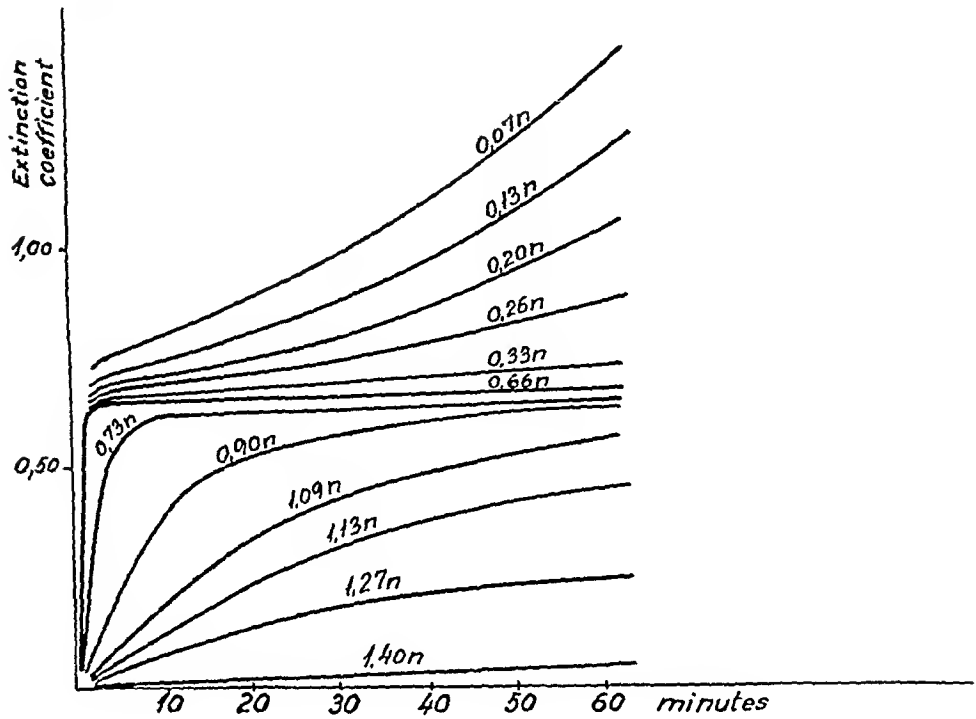

Fig.

It will be seen that the colour development is almost the same whether the solutions of amidol or molybdate are freshly made or old, although the older solutions have a tendency to cause a more intense colour. But it must be pointed out that old amidol solutions sometimes give erroneous results. They seem to be serviceable for one week when stored in a cold, dark place, but they should be discarded when the solution turns slightly yellow. With the molybdate solutions, on the other hand, the age seems to have practically no influence on the result.

4) The *temperature* is an important point, the colour intensity — as was to be expected — increasing rapidly with a rising temperature. If we stop heating (water bath 60° C.) an amidol-molybdate-phosphate solution (0.66 n H_2SO_4) after a certain time, *e. g.* after 10, 15, 20, or 30 minutes, the blue colour produced will when the mixture has been cooled remain constant for at least $3\,^1/_2$ hours. When determining very small quantities of phosphorus we obtained good results by heating the colour mixture to 60° C. for 15 minutes and then cooling it for 10 minutes. In this way the sensitivity was increased 55 % in proportion to colorimetric determination without previous heating.

Conclusion.

By colorimetric determination of phosphorus with Amidol (Agfa) and ammonium-molybdate, the acidity and temperature are of the greatest importance to the colour development. The best colour curve is obtained with a reaction mixture of 0.66 n in sulphuric acid, and by heating to 60° C. the sensitivity may be increased by more than 50 per cent. Of less importance are the appearance (age) of the amidol powder and the freshness of the amidol and molybdate solutions. The amidol solution should, however, be stored in a cold, dark place and should not be used longer than about 1 week.

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The Formation Mechanism of Oestrogenic Hormones.

III. Lipids of the Pregnant Rabbit Ovary and their Changes at Gonadotropic Stimulation.[1]

By

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There is at present little clear evidence as to the intimate nature of the ovarian biochemical processes involved in the formation of oestrogenic hormones. It has long been recognized, however, as a result of several histological investigations, that the cells of the theca interna and of the interstitial gland contain abundantly great quantities of lipids. Analyses carried out on ovaries of pregnant rabbits show that lipids may comprise even 15 per cent of the wet weight (corresponding to a lipid content of 50 per cent calculated on dry weight). These lipids are mainly composed of free and esterified cholesterol, phospholipids and residual fatty acids. The fact that this glandular cell system — the only known function of which is the formation of oestrogenic hormones — may contain such a great amount of lipids, suggests that these lipids probably play an important rôle in the biochemical processes involved in the formation of the hormones. This assumption directed our interest to *the lipid metabolism of the ovarian interstitial gland during the formation of oestrogenic substances*. As an introductory part of our investigations, here we report changes in the lipid constituents of the rabbit ovary 24 hours after the administration of PMS or PU,[2] which in a few

[1] Aided by a grant from Statens Medicinska Forskningsråd.

[2] A.B. Leo, Hälsingborg, has kindly assisted us in our investigations by placing its preparations Antex and Physex Leo at our disposal.

hours elicit an intensive hormone production in the almost resting cell system. In a following paper the detailed course of these changes (during the first 48 hours) will be published.

The investigations are based upon the fact that during certain sexual phases the rabbit ovary is nearly exclusively made up of a uniform interstitial gland with a quantitatively unimportant follicle system, and that this latter has quite a low lipid content compared to that of the interstitial gland.

These facts offer a very favourable field of investigation as *conditions are given to examine the behaviour of an isolated cell system, rich in lipids, the biochemical activity of which experimentally can be regulated by gonadotropic stimulation.*

Material and Methods.

The material is divided into three groups. In the first series ovaries of pregnant rabbits were examined before and 24 hours after the intravenous administration of 450 I. U. PMS or PU. The second group differed only in hypophysectomized animals being used. In the last series ovaries of oestrous rabbits were investigated immediately after coitus and 6 hours later.

With regard to the considerably great variations in the amount of the lipid constituents of different animals it seemed advisable to establish the changes by comparing the two ovaries of the same animal. Hence the first ovary of the animals was extirpated in *Citodon-Na* (Leo) anaesthesia, followed immediately by the intravenous injection of the gonadotropic hormone. After the elapse of 24 hours the other ovary was removed. In the third series the extirpation of the first ovary was carried out 10—15 min. after coitus, while the other one was removed 6 hours later.

Immediately after the extirpation of the ovaries the greater Graafian follicles were punctured, all of the corpora lutea removed and the ovaries weighed. Then the ovaries, composed almost exclusively of interstitial gland, were ground in a mortar with sand until a thin paste was formed. This paste was extracted with a 3 : 1 mixture of ethanol and peroxide-free ether, boiling for 5 minutes. The alcohol-ether extract was then filtered into a 100 ml. volumetric flask, and the paste extracted once more with another portion of the solvent. After filtration the two extracts were combined, cooled and made to volume. For all of the analyses various aliquots of this extract were used.

Often two ovaries were combined, one extract representing in this way two ovaries from different animals in order to diminish — with the same number of determinations — the rôle of the biological variation in the evaluation of the results.

Hypophysectomy was carried out by the parapharyngeal route. In order to control the completeness of the hypophysectomy the tissues

in the neighbourhood of the operation area were histologically examined.

The animals were kept on a common diet, containing hay, corn and turnips.

Analyses of the lipid constituents:

1. Total cholesterol was determined by digitonin precipitation after saponification as described by KELSEY (1939).

2. Free cholesterol was determined in the phospholipid-free acetone extract (see p. 3) by precipitation with digitonin according to KELSEY. In both cases the modified Liebermann-Burchard reagent was used as proposed by SPERRY and BRAND (1943).

3. Phospholipids were determined after $MgCl_2$-acetone precipitation by the oxidative micromethod of BLOOR (1929, 1943). This method gives the same values for phospholipids as the determination of the phospholipid-P (GORTNER, 1945).

4. Total fatty acids: oxidative determination according to BLOOR (1928, 1943).

5. Acetalphospholipids were determined as plasmal with the fuchsin reagent (Merck's fuchsin) of FEULGEN and GRÜNBERG (1939) and FEULGEN and BERSIN (1939). In order to reduce the "pseudocolour" (Pseudofärbung), the colorimetric estimations were carried out immediately after shaking out the dye with amylalcohol.[1]

All the colorimetric determinations were carried out with a Spekker photoelectric colorimeter.

The change in dry weight as a consequence of gonadotropic stimulation was examined as follows: the ovaries were cut into small pieces and extracted continuously with ether in a Soxleth apparatus for 24 hours. After having been dried in an oven at 120° C. for two hours, the dry material was weighed and the total nitrogen determined according to the Micro-Kjeldahl procedure.

I. Lipids of the Normal Rabbit Ovary.

We have but a very few informations concerning the normally occurring lipid constituents and their functional variations in the ovarian interstitial gland. It has been shown by BOYD (1935) that certain lipids of the *whole* ovary undergo great quantitative changes in the pregnant rabbit. However, it is hardly possible to gather any definite conclusions from this and other (1936) data of BOYD concerning the lipids of the *interstitial cells* and their relationship to the formation of hormones in the ovary, as the chemical analyses carried out by him include the lipids of both

[1] Recently ANCHEL and WAELSCH (1944) described a more suitable method to avoid the formation of the "pseudocolour" by the use of capryl alcohol. Unfortunately this solvent was not available during the course of our investigations.

the interstitial gland and the corpora lutea (The latter may constitute $^1/_3$ of the whole ovary.) It is obvious that these two ovarian constituents are responsible for the formation of quite different hormones, and thus their united analysis has only a very limited value with regard to the lipids of the interstitial gland. In addition we have to bear in mind that the production of hormones during pregnancy is at present imperfectly understood.

Sterols: In the first hand interest has been focused on the sterol found in the interstitial gland and interpreted in our previous papers (CLAESSON and HILLARP, 1946, 1947) as the precursor of the oestrogenic hormones. The digitonin precipitability, the positive Liebermann-Burchard reaction and the negative keto-reactions gave a well founded evidence to our previous assumption that this substance may be identical with cholesterol or with some closely related substance. As it is of primary importance for the biochemical interpretation to elucidate with certainty the real nature of this sterol, attempts have been made to identify this substance. Our method was briefly as follows:

The lipids of 50 pregnant rabbit ovaries were extracted with chloroform, the extract evaporated in N_2 and the residue taken up in petroleum ether. This solution was chromatographed on Al_2O_3 according to BROCKMAN. The esterified sterol fraction was eluted with 10 per cent ethylether in petroleum ether, and the free sterol fraction with 10 per cent ethanol in petroleum ether (HESS, 1947). After saponification of the esterified substance with potassium hydroxide in methanol and recrystallisation three times from ethanol the substance showed the following properties:

1. Rhombic needle crystals.
2. Melting point: 148° C. No melting point depression with cholesterol (Merck).
3. $[\alpha]_D^\circ = -39°$ in chloroform.
4. Bromine number: equivalent to one double bond.
5. Precipitable with digitonin.
6. No reaction with Girard's reagent T.
7. No characteristic UV absorption.

The non esterified sterol fraction showed the very same characteristics. — According to these analytical data the sterol investigated is identical with cholesterol.

Our experiments, however, do not exclude the coexistence of other sterols or even steroids in the ovarian interstitial gland, but they present sufficient evidence to the statement that cholesterol is the substance which dominates the sterol fraction of the

pregnant ovary. Hence during the course of the following investigations this fraction will be considered as composed exclusively of cholesterol.

In addition it must be recalled here that the amount of steroids, possessing oestrogenic activity, present in the pregnant rabbit ovary, is so slight that even the total lipid extract of a whole ovary fails to induce any vaginal reactions in castrated mice (CLAESSON and HILLARP, 1947 a).

As can be seen from Tables I and III, the cholesterol content may show considerable variations; in a group of 7 oestrous rabbits the total cholesterol varied between 7.8 and 10.8 mg./g. (average 9.4), the ester cholesterol between 4.2 and 7.0 (av. 6.0) while the free cholesterol varied between 2.5 and 4.0 mg./g. (av. 3.4). The same technique gave the following values for a group of 16 pregnant rabbits (6—8th day of pregnancy): 11.2—31.5 (average: 21.0), 8.8—28.6 (av. 16.9) resp. 2.2—4.6 (av. 3.3 mg./g.).

Thus the accumulation of sterols during the first phase of pregnancy previously observed by us (1947 a) is due to the increased storage of esterified cholesterol.

Phospholipids: No attempts were made to analyse particularly the various components in the phospholipid fraction. The phospholipids precipitated by acetone were completely soluble in moist ether, not having any unsoluble sphingomyelin residue.

As is shown in Tables I and III, the values for phospholipids show a smaller deviation from the average than those for cholesterol. Furthermore there is no significant difference between the oestrous and the pregnant groups; the average for 8 oestrous animals being 24.3 mg./g., while the same for 16 pregnant rabbits is 26.4 mg./g. — From a quantitative point of view the phospholipid content of the ovary does not differ considerably from that of other organs, viz. that of liver, adrenals, kidneys etc. (See PAGE, PASTERNAK and BURT (1931), MACLACHLAN, HODGE and WHITEHEAD (1941).)

Acetalphospholipids: For two reasons it was necessary to estimate the acetalphospholipids: first because of the relative abundance of the interstitial gland in acetalphospholipids (shown with histochemical reactions by FINK (1941) and KOCH (1941)) and secondly the generally assumed but hitherto experimentally not confirmed rôle of these substances in the intermediary fat metabolism (EHRLICH and WAELSCH, 1946). — The values — calculated as plasmal — obtained in a group of 8 oestrous rabbits vary between 0.20—

Table

Lipid changes in the ovarian interstitial gland of normal pregnant rabbits the intravenous administration of 450 I. U. PMS (in case of rabbit No. 7: lipid content of the stimulated ovary is calculated as well on the wet weight 194 (2). Acetal phospholipids

Animal No.	Day of Pregnancy	Weight of the Ovaries mg.	Total Cholesterol mg./g.		Ester Cholesterol mg./g.	
			1	2	1	2
1	6	L 250	28.3		—	
2		L 395				
1		R 450	6.5	5.3	3.9	3.2
2		R 490	—77 %	— 81 %		
3	6	L 420	26.7		23.0	
4		L 210				
3		R 705	6.4	4.9	3.5	2.7
4		R 315	— 76 %	— 82 %	— 85 %	— 89 %
5	6	L 165	15.2		11.0	
6		L 330				
5		R 205	7.1	6.1	4.4	3.8
6		R 450	— 54 %	— 60 %	— 60 %	— 65 %
7	8	L 255	17.3		12.7	
7		R 375	5.5	4.4	1.6	1.3
			— 69 %	— 74 %	— 88 %	— 90 %
8	6	L 245	31.5		28.8	
8		R 320	5.7	5.0	3.3	2.8
			— 82 %	— 84 %	— 89 %	— 90 %
9	6	L 310	18.7		15.5	
9		R 570	4.2	3.0	1.3	0.9
			— 78 %	— 84 %	— 92 %	— 94 %
10	6	L 320	19.1		16.9	
10		R 480	2.8	2.3	1.3	1.0
			— 85 %	— 88 %	— 93 %	— 95 %
11	6	R 615	11.2		8.8	
12		R 450				
11		L 940	7.3	5.8	4.9	3.9
12		L 710	— 35 %	— 48 %	— 44 %	— 56 %
13	6	R 400	18.4		15.6	
14		R 350				
13		L 540	7.7	6.1	5.1	4.0
14		L 610	— 58 %	— 67 %	— 68 %	— 74 %
15	7	R 280	23.7		20.2	
16		R 180				
15		L 350	6.7	5.9	3.7	3.2
16		L 250	— 72 %	— 76 %	— 82 %	— 84 %
17	6	R 425				
17		L 540				
Average:			— 69 %	— 74 %	— 78 %	— 82 %

I.

at gonadotropic stimulation. The first ovary extirpated immediately before 450 I. U. PU), the other one removed 24 hours after the injection. — The of the first ovary (1) as on its own wet weight reduced as described on page calculated as plasmal.

Free Cholesterol mg./g.		Phospholipids mg./g.		Residual Fatty Acids mg./g.		Acetalphospholipids mg./g.	
1	2	1	2	1	2	1	2
—		32.0		44.4			
2.6	2.2	40.9 + 27 %	33.4 + 4 %	37.1 — 17 %	30.3 — 32 %		
3.7		34.0		46.4			
2.9 — 22 %	2.2 — 41 %	44.9 + 33 %	35.0 + 3 %	33.0 — 29 %	25.2 — 46 %		
4.2		26.0		46.2			
2.6 — 38 %	2.3 — 45 %	35.9 + 37 %	30.7 + 18 %	19.8 — 58 %	17.0 — 63 %		
4.6		27.1		30.6			
3.9 — 17 %	3.2 — 30 %	40.4 + 49 %	32.3 + 19 %	18.0 — 41 %	14.6 — 52 %		
2.9		26.0		16.7			
2.5 — 14 %	2.1 — 28 %	32.2 + 25 %	28.2 + 9 %	16.7 0 %	14.6 — 13 %		
3.2		25.0		29.7			
2.9 — 10 %	2.0 — 38 %	42.3 + 72 %	29.9 + 19 %	16.1 — 46 %	11.4 — 62 %		
2.2		23.0		24.4			
1.6 — 29 %	1.3 — 41 %	28.0 + 19 %	22.3 — 3 %	18.7 — 23 %	15.0 — 38 %		
2.4		22.4		42.3		0.28	
2.3 — 4 %	1.9 — 20 %	31.2 + 34 %	24.4 + 9 %	20.9 — 51 %	16.5 — 61 %	0.26 — 7 %	0.21 — 25 %
2.8		25.8		41.6		0.28	
2.7 — 5 %	2.1 — 25 %	31.5 + 22 %	24.8 — 4 %	31.7 — 24 %	25.1 — 40 %	0.28 0 %	0.22 — 21 %
3.5		22.8		37.0		0.35	
3.0 — 13 %	2.6 — 26 %	38.7 + 70 %	33.6 + 48 %	22.4 — 39 %	19.4 — 48 %	0.35 0 %	0.30 — 14 %
						0.35 0.30 + 14 %	0.35 0 %

Table

Lipid changes in the ovarian interstitial gland of hypophysectomized
the same as in Table I (to the animals No. 23 and No. 24 450

Animal No.	Day of Pregnancy	Weight of the Ovaries mg.	Total Cholesterol mg./g.		Ester Cholesterol mg./g.	
			1	2	1	2
18 18	12	L 255 R 425	— 6.7	5.0	— 3.5	2.6
19 19	12	L 225 R 360	19.1 —	—	15.2 —	—
20 20	12	R 435 L 530	9.8 4.6 — 54 %	4.2 — 57 %	7.0 2.5 — 65 %	2.3 — 67 %
21 21	13	L 270 R 340	16.7 11.5 — 31 %	10.2 — 39 %	14.5 8.9 — 38 %	7.9 — 46 %
22 22	7	L 360 R 470	13.9 5.0 — 64 %	4.3 — 69 %	11.1 2.8 — 75 %	2.4 — 78 %
23 24 23 24	10	L 75 L 110 R 85 R 170	18.4 9.2 — 50 %	7.7 — 58 %	14.7 5.4 — 64 %	4.5 — 69 %
Average:			— 50 %	— 56 %	— 61 %	— 65 %

0.35 mg./g. fresh weight (av. 0.28 mg./g.),[1] while in another group containing 7 pregnant rabbits (6—8th day of pregnancy) they are between 0.28—0.35 mg./g. (av. 0.31). As no standard was available for the colorimetric estimations, the absolute values must be taken with a certain reservation. On the other hand these values are — obviously — suitable for the judgement of differences — if any — due to gonadotropic stimulation.

Cerebrosides. Investigations on the presence of cerebrosides in the pregnant rabbit ovary were carried out with the modification of KIMMELSTIEL's method (1929), as described by KIRK (1938). In a total lipid extract corresponding to 200 mg. fresh ovary, no cerebrosides could be demonstrated. As these samples contained

[1] In 6 of these 8 animals only acetalphospholipids were determined. Thus the values obtained in these animals are not included in Table III.

II.

pregnant rabbits at gonadotropic stimulation. Treatment and calculation I. U. PU was administered, to the others 450 I. U. PMS).

Free Cholesterol mg./g.		Phospholipids mg./g.		Residual fatty acids mg./g.		Acetalphospholipids mg./g.	
1	2	1	2	1	2	1	2
3.5 3.1 — 11 %	2.4 — 31 %	34.1 48.7 + 43 %	36.5 + 7 %	— 63.5	47.6	0.24 0.28 + 17 %	0.21 — 12 %
3.9 2.4 — 38 %	2.1 — 41 %	26.7 39.5 + 48 %	30.7 + 15 %	121.8 —	—	0.31 0.31 0 %	0.24 — 23 %
2.8 2.1 — 25 %	1.9 — 32 %	33.6 40.5 + 21 %	36.7 + 9 %	115.6 62.3 — 46 %	56.5 — 51 %	0.16 0.21 + 31 %	0.19 + 19 %
2.2 2.6 + 17 %	2.3 + 5 %	27.0 61.5 + 128 %	54.4 + 102 %	167.8 58.5 — 65 %	51.8 — 69 %	0.19 0.19 0 %	0.16 — 16 %
2.8 2.2 — 21 %	1.9 — 32 %	36.7 42.5 + 16 %	36.9 0 %	58.6 36.1 — 38 %	31.3 — 47 %		
3.8 3.8 0 %	3.2 — 16 %	31.9 36.2 + 14 %	30.5 — 4 %	73.5 35.6 — 51 %	30.0 — 59 %		
— 13 %	— 24 %	+ 45 %	+ 21 %	— 50 %	— 56 %	+ 12 %	— 8 %

5—7 mg. of phospholipids, their cerebroside content ought to be at least 50 times lower than the phospholipid content (the smallest amount of cerebrosides which could be determined with the method used being 0.1 mg. per sample). Thus the ovary differs significantly in its cerebroside content from most of the other organs (with the exception of the liver), which have a considerably higher concentration of cerebrosides (KAUCHER, GALBRAITH, BUTTON and WILLIAMS, 1944; WILLIAMS, GALBRAITH, KAUCHER, MOYER, RICHARDS and MACY, 1945).

Residual fatty acids. One of the most remarkable characteristics of the interstitial gland — besides the high ester cholesterol level — is its richness in residual fatty acids (calculated as follows: 2/3 weight of the phospholipids + 2/3 weight of the esterified cholesterol are subtracted from the amount of total fatty acids). As is

Table III.

Lipid changes in the ovarian interstitial gland of oestrous rabbits at gonadotropic stimulation. The first ovary extirpated 10—15 minutes after coitus, the other 6 hours later. (Calculation of the lipid content as in Table I.)

Animal No.	Weight of the Ovaries mg.	Total Cholesterol mg./g. 1	Ester Cholesterol mg/g. 1	Free Cholesterol mg/g. 1	Phospholipids mg./g. 1	Residual fatty acids mg./g. 1	Acetalphospholipids mg./g. 1
25	L 115	10.8	6.8	4.0	24.9	111.1	
26	L 255						
25	R 195	8.1	4.6	3.5	30.8	77.8	
26	R 380	— 25 %	— 32 %	— 13 %	+ 24 %	— 30 %	
27	L 195	9.5	7.0	2.5	20.9	47.9	
28	L 160						
27	R 200	7.0	5.1	2.0	25.4	36.3	
28	R 215	— 26 %	— 27 %	— 20 %	+ 22 %	— 24 %	
29	L 135	7.8	4.2	3.6	19.4	—	
30	L 120						
31	L 105						
29	R 180	7.0	3.6	3.3	23.1		
30	R 170						
31	R 120	— 10 %	— 14 %	— 8 %	+ 18 %		
32	L 245	—	—	—	31.9	—	0.20
32	R 260	—	—	—	44.1	—	0.20
					+ 38 %		0 %
33	L 135						0.21
34	L 200						0.24
33	R 265						
34	R 240						+ 15 %
	Average:	— 20 %	— 24 %	— 14 %	+ 26 %	— 27 %	

shown in Tables I and III, the values for 4 oestrous rabbits are 47.9 and 111.1 (average: 79.5), and those for 16 pregnant rabbits are between 16.7 and 46.4 mg./g. (av. 35.9 mg./g.).

II. Changes in the Lipids at Gonadotropic Stimulation.

The first signs of gonadotropic stimulation manifest themselves in a marked hyperaemia and swelling of the ovary (See for instance ZONDEK and SULMAN, 1945, 1947). The hyperaemia is paralleled by a great increase in weight and this fact makes it very difficult to calculate the lipid concentration after the stim-

Table IV.

Blood lipids of the pregnant rabbit.

Animal No.	Day of Pregnancy	Total Cholesterol mg./100 ml.	Ester Cholesterol mg./100 ml.	Free Cholesterol mg./100 ml.	Phospholipids mg./100 ml.	Residual fatty acids mg./100 ml.
I	14	58	10	48	156	75
II	14	50	6	44	146	44
III	14	56	14	42	146	61
IV	19	50	8	42	204	136
V	19	46	2	44	166	72
	Average:	52	8	44	164	78

ulation. As the increase in weight is partly due to hyperaemia and presumably partly — as a consequence — to a tissue fluid accumulation, there is no more possibility to calculate the lipid content per g. of fresh ovary without any special considerations. On the other hand, if the two factors mentioned above would dominate, this fact could make it possible to express the lipid content even of these ovaries calculated on basis of the original wet weight (*i. e. before* stimulation). Using this mode of calculation, only an unimportant error could be introduced even if the whole increase in weight were solely due to hyperaemia. This statement becomes obvious when taking into consideration the fact that the lipid concentration in the blood is very low compared to that of the ovarian interstitial gland (Table IV).

As the original weight of the stimulated ovary is unknown, the lipid content of this must be calculated on the basis of the ovary first removed. In this way the possible error due to the initial difference in the weight of the two ovaries of a single animal can be compensated by the comparison of the total amount of stimulated and non-stimulated normal ovaries in a larger group of test animals. The correctness of this calculation is demonstrated in Table V, representing the weights of both the ovaries of 12 pregnant rabbits.

The mode of calculation described above is based on the assumption that the gonadotropic stimulation does not cause any noticeable increase in the cell number of the interstitial gland. In

Table V.

Weights of both ovaries in untreated pregnant rabbits.

Animal No.	Day of Pregnancy	Weight of the Ovaries mg.		Difference in Weight Between Left and Right Ovary Per cent
		Left	Right	
Pr 1.....	12	320	270	—16
Pr 2.....	12	270	260	— 4
Pr 3.....	12	190	220	+15
Pr 4.....	12	320	310	— 3
Pr 5.....	18	275	290	+ 5
Pr 6.....	18	220	230	+ 7
Pr 7.....	15	360	315	—12
Pr 8.....	13	435	480	+10
Pr 9.....	13	205	175	—15
Pr 10.....	13	475	500	+ 5
Pr 11.....	17	495	545	+10
Pr 12.....	17	315	280	- -11
			Average:	± 0

order to support evidence to this suggestion dry weight measurements were carried out on both the ovaries of a group of 5 rabbits before and 24 hours after the administration of 450 I. U. PMS (Table VI). The increase in wet weight varied between 58 and 96 per cent (av. 67 per cent) and the corresponding increase in dry weight between 28 and 49 per cent (av. 38 per cent). As the dry weight of the normal ovary constitutes 15.3 per cent of the fresh weight, an increase in the number of cells consequently may represent maximally the half — and probably considerably less — of the increase in wet weight.[1]

Therefore the lipid content is given in the Tables also in g. per fresh weight *after* stimulation, by which, however, the weight increase has been reduced with 50 per cent. In this way we took into consideration a maximal increase in the number of the cells, representing 50 per cent of the increase in fresh weight.

The changes in the lipids of the normal rabbit ovary at gonadotropic stimulation are summarized in Table I. Above all the amount of esterified cholesterol decreases markedly (44—93 per cent; av. 78 per cent), while the free cholesterol shows only a moderate decrease (4—38 per cent; av. 17 per cent). *It is thus the stored ester cholesterol, which is above all utilized for the formation of oestrogenic hormones.*

[1] Calculating with a dry weight of 7 per cent for the blood and tissue fluid in the ovary, these two may represent 80 per cent of the weight increase.

However, these values do not decrease below a certain basal level, in spite of the intensive gonadotropic stimulation (av. 3.3 mg./g.) not even in the case of a continued stimulation (see Table VII); hence the remaining amount may be interpreted as the cholesterol of the cell's own, which does not take part in the formation of oestrogenic substances. *Thus the applied gonadotropic stimulation probably mobilises all the cholesterol stored as precursor.*

In addition the phospholipids are markedly increased (22—72 per cent; av. 39 per cent), if calculated regardless to the possibility of an increase in the cell number. But taking into consideration the probability of an increase in protoplasm which may constitute 50 per cent of the increase in weight, as mentioned above, it becomes at once questionable whether or not the phospholipids of a single cell do show any real increase. Only a detailed analysis of the correlation between the changes in wet weight, dry weight, and phospholipid content following gonadotropic stimulation may give a definite answer concerning the adequate mode of calculation. Hence the discussion of this problem will be deferred to a following paper.

The residual fatty acids show a considerable decrease (average: 33 per cent). The question whether, at gonadotropic stimulation, these fatty acids undergo oxidative breakdown, or are utilized for the synthesis of newly formed phospholipids can, however, not be answered here.

The values for acetalphospholipids before and 24 hours after the administration of PMS were the same through the course of this study; thus the possibility of an increase in the acetalphospholipids due to gonadotropic stimulation can with certainty be excluded. On the other hand there is a certain probability of a slight decrease in acetalphospholipids due to an increase in number of the interstitial cells.

The lipid changes at gonadotropic stimulation were also examined in hypophysectomized pregnant rabbits in order to support evidence to the assumption that PMS or PU exerts its effect by acting directly upon the ovaries and not via the pituitary body of the animal and furthermore that the normally occurring lipid accumulation in the ovary during pregnancy exerts no important influence on the results obtained. As may be seen from Table II, the lipid changes in the ovaries of the hypophysectomized animals parallel with those of the group with intact pituitary (ester cholesterol: — 61 per cent, free cholesterol: — 13 per cent, phospho-

Table

Changes in the wet weight, dry weight and total nitrogen content of pregnant before the administration of 450 I. U. PMS, the right ovary 24 hours lipid-free

Animal No.	Day of Pregnancy	Wet Weight in Mg.		
		Left Ovary	Right Ovary	Increase in Weight Per cent
IV	19	324	521	61
V	19	451	720	60
VI	18	318	503	58
X	8	360	705	96
XI	12	435	705	62
	Average:			67

lipids: + 45 per cent, and residual fatty acids — 50 per cent in average).

At last the lipid changes due to gonadotropic stimulation were also investigated in oestrous rabbits 6 hours post coitum. *This experiment clearly shows* (Table III) *that — from a principle point of view — the gonadotropic hormone produced by the animals' own pituitary exerts the same effect on the ovarian lipids as PMS or PU.*

Discussion.

Long and his coworkers (Sayers, Sayers, White and Long, 1943; Sayers, Sayers, Fry, White and Long, 1944; Sayers, Sayers, Lewis and Long, 1944; Sayers, Sayers, Liang and Long, 1945, 1946) were the first to direct interest on the relationship between the cholesterol content and the formation of steroids in adrenals. Similar experiments were carried out also by Popják (1944) and Abelin (1944). In spite of the numerous difficulties presented by the complex structure of the adrenals and by the complicated formation mechanism of the adrenocortical hormones, already their results offer evidence to assume that cholesterol might be transformed into steroid hormones. The direct evidence has been presented by Bloch (1945), showing that deuteriocholesterol administered to a pregnant woman was transformed into pregnandiol. Furthermore the conditions for the storage of cholesterol in the corpora lutea of rats were analysed

VI.

ovaries at gonadotropic stimulation. The left ovary extirpated immediately after the injection. Values for dry weights and total nitrogen refer to the material.

Dry Weight in Mg.			Dry Weight Per cent of Wet Weight		Total Nitrogen Per cent of Dry Weight	
Left Ovary	Right Ovary	Increase in Weight Per cent	Left Ovary	Right Ovary	Left Ovary	Right Ovary
50.4	64.6	28	15.3	12.5	13.74	13.10
67.8	90.6	34	15.1	12.6	13.33	13.62
48.8	68.0	39	15.3	13.6	13.48	13.36
57.5	85.8	49	15.9	12.2		
64.0	88.2	38	14.7	12.5		
		38	15.3	12.7	13.51	13.36

by EVERETT (1945, 1947). Finally this paper and our previous publications (CLAESSON and HILLARP, 1946, 1947; ALDMAN, CLAESSON, HILLARP and HÖGBERG, 1948) clearly show that the biosynthesis of oestrogenic substances in the ovary is based on the transformation of cholesterol, and that for this purpose in the first hand the esterified cholesterol stored in the cells will be mobilised.

Table VII.

Changes in the cholesterol content of the interstitial gland (pregnant rabbit ovaries) at intense and prolonged gonadotropic stimulation. The left ovary extirpated immediately before the administration of 450 I. U. PMS; after 24 hours this dose was repeated and after further 24 hours the right ovary removed. (Concerning the mode of calculation see Table I.)

Animal No.	Day of Pregnancy	Weight of the Ovaries mg.	Total Cholesterol mg./g.		Ester Cholesterol mg./g.		Free Cholesterol mg./g.	
			1	2	1	2	1	2
I.....	17	L 285 R 370	34.0 6.7 —80 %	5.8 —83 %	30.2 3.5 —88 %	3.0 —90 %	3.8 3.2 —16 %	2.8 —26 %
II....	13	L 300 R 425	25.0 6.3 —75 %	5.3 —79 %	21.0 3.0 —86 %	2.5 —87 %	4.0 3.3 —18 %	2.8 —30 %
III...	12	L 360 R 440	28.9 5.0 —83 %	4.4 —85 %	25.4 2.3 —91 %	2.0 —92 %	3.5 2.7 —23 %	2.4 —32 %

Hence cholesterol may be considered as a general precursor of the steroid hormones. Thus the first phase of the investigations upon the formation mechanism of steroids seems to be finished. However, the most fundamental question, *i. e.* the elucidation of the biochemical processes responsible for the transformation of cholesterol into hormones still remains an unsolved problem. The hereby presented paper is only a first introductory attempt to throw light upon this problem. There is yet, however, no possibility to put these results into closer relation to the formation of oestrogens. Only a detailed analysis of the various phases in the lipid metabolism may clear up this problem. In addition it should be emphasized that in the further study of the biochemical reactions, involved in the biosynthesis of oestrogenic hormones, considerable advantage is offered by the isolated cell system of the ovarian interstitial gland. Working with this uniform cell system it seems to be possible to map down these metabolic processes, which may result in a better understanding of the formation mechanism of oestrogenic hormones.

Summary.

The interstitial cells of the ovary — like other steroid-producing cells — exhibit a particularly high lipid content (up to 15 per cent of the fresh ovary). This fact suggests that the lipids play an important rôle in the oestrogen producing function of these cells. It seemed therefore of interest to study the lipid metabolism of the ovarian interstitial gland during the formation of oestrogenic substances.

As a first introductory part of these investigations data are here reported concerning the lipid constituents occurring in the normal rabbit ovary and their changes 24 hours after the administration of 450 I. U. PMS or PU (both to normal and hypophysectomized animals), and 6 hours post coitum.

The main lipid constituents of the interstitial gland are cholesterol (stored chiefly as ester cholesterol), phospholipids, acetalphospholipids and residual fatty acids, while the presence of cerebrosides could not be demonstrated. — A closer analysis shows that the sterol interpreted in previous papers as the precursor of oestrogenic hormones is identical with cholesterol.

At gonadotropic stimulation (during 24 hours) the content of

esterified cholesterol decreases markedly (average: 78 per cent for normal and 61 per cent for hypophysectomized animals), whereas the free cholesterol shows only a slight decrease (17, resp. 13 per cent). The applied gonadotropic stimulation probably mobilises all the cholesterol stored as precursor. Coincidently the content of phospholipids greatly increases (38, resp. 45 per cent) when calculated regardless of the possibility of an increase in the number of the cells (See discussion on page 194). The amount of acetalphospholipids seems not to be influenced by gonadotropic stimulation. Finally the gonadotropic stimulation causes a marked decrease in the residual fatty acid content (33, resp. 50 per cent).

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A Method for the Determination of Carboxy-Haemoglobin Concentrations by Analysis of the Alveolar Air.

By

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The formation of carboxy-haemoglobin during inhalation of carbon monoxide occurs according to the formula $\frac{COHb}{O_2Hb} = \frac{MpCO}{pO_2}$. If the CO-tension in the blood is greater than that in the alveolar air, carbon monoxide is released by the blood on its passage through the lungs until equilibrium is reached. This reaction is influenced by the temperature of the blood and by the constant, M, which according to some authors varies in different individuals. Careful determinations made by SENDROY, LIU and VAN SLYKE (1929) show that this factor probably is the same for different members of the same animal species, and in a human being = 210 ± 2.5.

The theoretical conditions exist, therefore, for the calculation of the COHb-concentration in the blood, if the CO-concentration in the alveolar air is known. Previously, this theory seems to have been tested only by HENDERSON and his associates (1921), who report from an investigation not only the COHb value, but also the CO-concentration in a sample of alveolar air. Their results do not, however, indicate a great degree of accuracy in their measurements.

Believing that the above theory could be applied in the development of a quick method for the determination of COHb-con-

centrations in the blood during suspected carbon monoxide poisoning, it was considered of value to subject it to further proof. This has led to the development of a simple and practical method for the determination of COHb.

Apparatus for the Measurement of Carbon Monoxide in Gas Mixtures.

When the value for M, found by SENDROY, LIU and VAN SLYKE (1929), is inserted in the formula for the formation of COHb, together with the accepted value for O_2-pressure in the lungs and the CO-pressure calculated at a concentration of 0.01 % in the alveolar air, the comparable value for COHb-concentration is found to be about 14 %. This agrees very well with determinations made by FORBES, SARGENT and ROUGHTON (1945) of the CO-uptake at different CO-concentration in the air. At 0.01 % they found that during equilibrium, carbon monoxide was absorbed to 14 % COHb.

Other investigators have found higher and even somewhat lower values, which can be explained by the difficulties in determining the COHb as well as the CO in the air, but might also be an indication that M is not absolutely constant for different individuals. However, it was apparent that if COHb-concentrations of about 1 % are to be calculated from an analysis of alveolar air specimens it was necessary to be able to measure CO-concentrations as low as 0.0005 %. Since no practical method for the determination of such low concentrations existed, it was necessary to construct an apparatus for this purpose.

A carbon monoxide indicator, capable of measuring CO-concentrations in the air of 0.01 %, had been constructed by engineer G. LINDELÖV. This apparatus, similar to earlier apparatus for the same purpose, is based on the principle of oxidizing carbon monoxide with the help of a catalyst (hopcalite) and measuring the liberated heat. In Lindelövs apparatus this measurement is made with a differential thermometer. With the cooperation of engineer Lindelöv this apparatus was reconstructed so as to increase its sensitivity 20 times. At the same time the required filters (for elimination of moisture, CO_2, alcohol, acetone) for alveolar air analysis were installed and tested. The results of this work are shown in Fig 1.

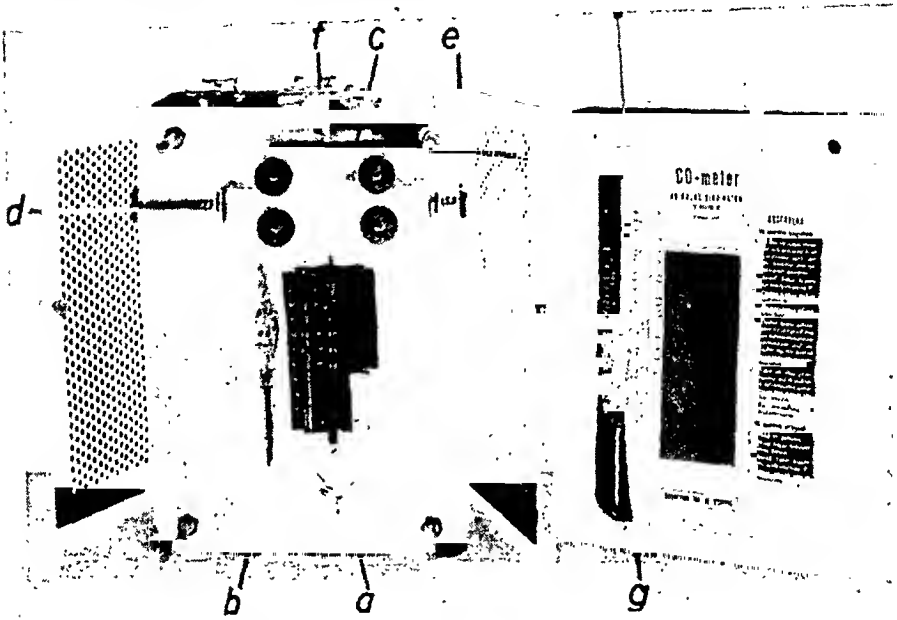

Fig. 1. The CO-meter constructed by LINDELÖV and SJÖSTRAND. The front removed and placed to the right. a. Differential thermometer. b. Liquid manometer for measuring velocity of air flow. c. Chamber containing differential thermometer ampoules, at left surrounded by chemically neutral substance, at right by "hopcalite". d and e. Stop-cocks for regulating air flow. f. Adjustment screw on the scale. g. Slide rule for calculations of CO and COHb values. To the left in the opened side the potassium hydroxide filter, on the opposite side the membrane pump.

Determinations are made by pumping the air specimen through the apparatus by means of a membrane pump having a capacity of at least 1 litre per minute, the air flow being regulated by the help of a liquid manometer. The heat liberated in the presence of carbon monoxide is read from the thermometer after 2—5 minutes, sometimes even later if the gas volume is sufficient. The precision increases to some extent with the length of time for the determination.

The effectivity of hopcalite is variable and it is therefore necessary to calibrate the apparatus with a gas sample having a fixed CO-concentration. Determinations made after 5 minutes are exact to $\pm$ 2 %. In order to attain a higher degree of accuracy it is necessary to make repeated calibrations.

Using the value obtained from the known CO-concentration in the calibrating gas and the measured value, the CO-concentration in the air specimen can be easily calculated with the help of a "slide rule" installed on the front of the apparatus.

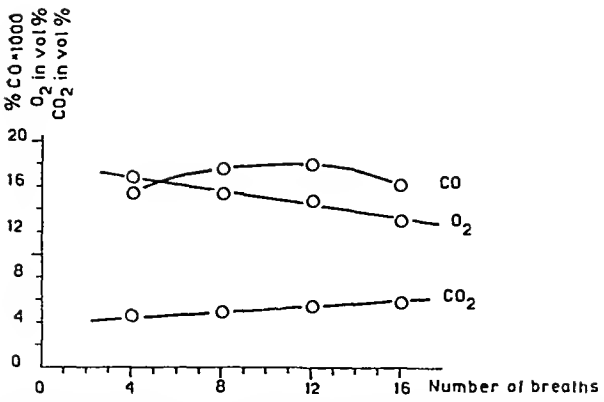

Fig. 2. Diagram of the CO-, O_2-, and CO_2-concentrations with different number of breaths in a rubber bag containing exhaled air. Test individual has absorbed CO for 12 % COHb.

Method for Getting Alveolar Air Samples.

For precise determinations of the CO-concentration with the above apparatus, it is necessary to have a gas specimen of about 5 litres. Such a large "alveolar air specimen" can be obtained by re-breathing 100 % O_2 until equilibrium is reached between CO-concentration in the specimen and in the lungs. A CO_2-filter must be used and the nitrogen in the lungs first be expired in order to prevent variations in the CO_2 and O_2 tension of the alveolar air. This method is very precise, but requires quite a little apparatus which renders it less suitable for practical use.

The method can be simplified by collecting the expired air in a rubber bag and than rebreathing in the bag several times. The alveolar O_2-concentration thereby falls, and the CO_2-concentration rises, which affects the COHb-dissociation. Fig. 2 is a diagram of the CO, O_2 and CO_2-concentrations in an experiment with different numbers of re-breathings in a rubber bag containing expired air. It is apparent that the CO-concentration first rises and then falls slowly. This latter being probably associated with the decreased O_2-pressure. It can be expected that if 10 ex- and inhalations are made in the bag, the error

Table 1.

CO-concentration in Alveolar Air Samples.

Test person	CO-concentrations in indicator units				
	Sample I	Sample II	Sample III	Sample IV	Sample V
S. J.	27¾	28	28¾	27½	—
A. G.	24½	23¾	24½	23½	24
T. B.	16	16	15½	15½	16½

Fig. 3. Arrangement for the collection of samples of alveolar air. See text.

will be relatively small, even with different lung and breath volumes. This can be seen in Table 1 which shows the results of repeated tests on some individuals who had absorbed carbon monoxide to give between 10 and 15 % COHb. The single determinations usually lie within $\pm$ 3 % of the mean value. However, it has been observed that this error may be decidedly greater if very rapid or deep breaths are taken. Therefore the individual was instructed to breathe steadily with ordinary breaths *i. e.* about 10 per 45 seconds.

An apparatus was constructed to simplify the technique for taking a sample of alveolar air. This consisted of a three-way stop-cock, one leg fitted with a valve, the second connected to a face mask and the third with a connecting piece for the rubber bag (Fig. 3). The stop-cock can be set in two positions. In one, the outside air is sucked in through the valve and blown out into the rubber bag. In the other both in- and exhalation take place in the bag.

Calculation of the COHb-concentration.

In accordance with the experimental results already mentioned and the theoretical calculations on the relation between the alveolar CO-concentration and the COHb-concentration, breathing of normal air with a CO-concentration of 0.01 % gives a COHb-concentration of about 14 % and, reversely by a COHb-concentration of 14 % the alveolar CO-concentration is 0.01 %. When the specimens are collected in the manner mentioned above the O_2 pressure falls, however, it was expected that 0.01 % CO in the alveolar gas sample would correspond to a higher COHb value. In comparisons between the measured CO value and the calculated COHb value during administration of known quantities of CO and even with direct spectrophotographically measured COHb values

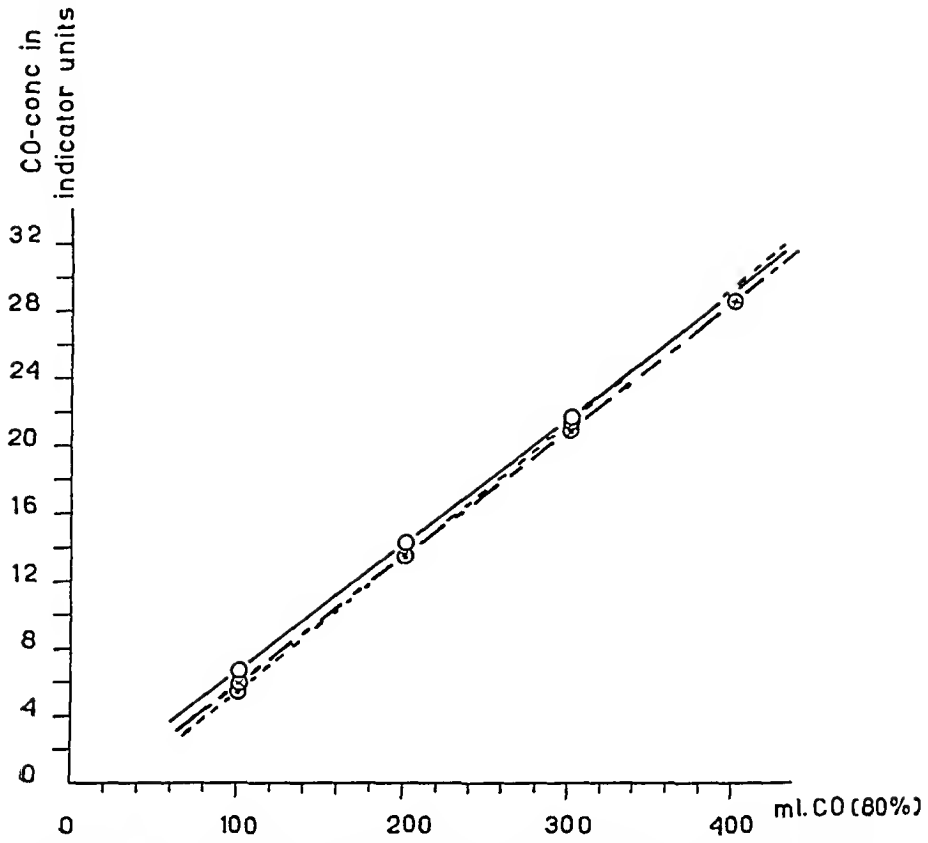

Fig. 4. Diagram of the CO-concentration in the alveolar air and the quantity of CO administered to three different individuals.

it was found that a CO-concentration of 0.01 %, in specimens collected as described, corresponds to about 16 % COHb.

In order to test whether there is a direct relation or not between the CO-concentration in the alveolar air and the COHb-concentration, different persons were allowed to breathe for 15 minutes in a Krogh spirometer supplied with a fixed quantity of carbon monoxide, in air, whereby practically all carbon monoxide was absorbed. Thereafter, an alveolar air specimen was taken for the determination of the CO. The spirometer was again supplied with the same quantity of CO and a new sample taken after 15 minutes. This was further repeated one or two times. The alveolar CO values so obtained have been compared to the administered quantity of CO in the diagram in Fig. 4. From this it is apparent that there is a direct relation between the alveolar CO-concentration and the quantity of CO administered. The highest COHb value in this and in other similar experiments was 40 %.

Since a direct relation seems to exist between the alveolar CO-concentration and the COHb-concentration, the latter may be easily calculated from the measured CO value by using as the basis the fact that 0.01 % CO corresponds to 16 % COHb. As already described a "slide rule" has been attached to the CO-meter for the simplification of the calculations, including the corrections for the sensitivity of the apparatus and for the calibrating gas.

The Errors of the Method.

The error in determination of the CO-concentration with the CO-meter and in collecting the samples of alveolar air has already been shown. In determinations on different individuals several other sources of error may arise, such as variations in lung volume, in O_2 consumption and in the dissociation constant for COHb. In order to test the significance of these and other sources of errors for the accuracy of the method, 23 different test indivuals were given a fixed quantity of carbon monoxide 3 times in succession as previously described. The alveolar CO-concentration before and after each administration was determined. The CO values obtained were then plotted in relation to the COHb-concentrations calculated from the known quantity of CO administered, and the volume of blood based on the body weight (7.8% in accordance with determinations with the carbon monoxide method by ASMUSSEN, 1945). The results are illustrated in Fig. 5. Fully 90 % of all values lie within $\pm$ 10 % of a mean, and this error per cent seems to be the same for both high and low values.

It is probable that the accuracy is greater than shown by these figures, since the volume of blood is not only relative to the body weight but shows considerable individual variations. Unfortunately, it was not possible to determine the errors in the method more carefully by comparison with direct measurements of the COHb in the blood. Measurements with the spectrophotographic method by well trained operators showed much greater variations not only between the different specimens from the same individual but also between the COHb values in different individuals in relation to the calculated value. This was particularly noticeable at low COHb values, due to the fact that the errors of determination in the blood method are to a certain extent independent of the absolute COHb value.

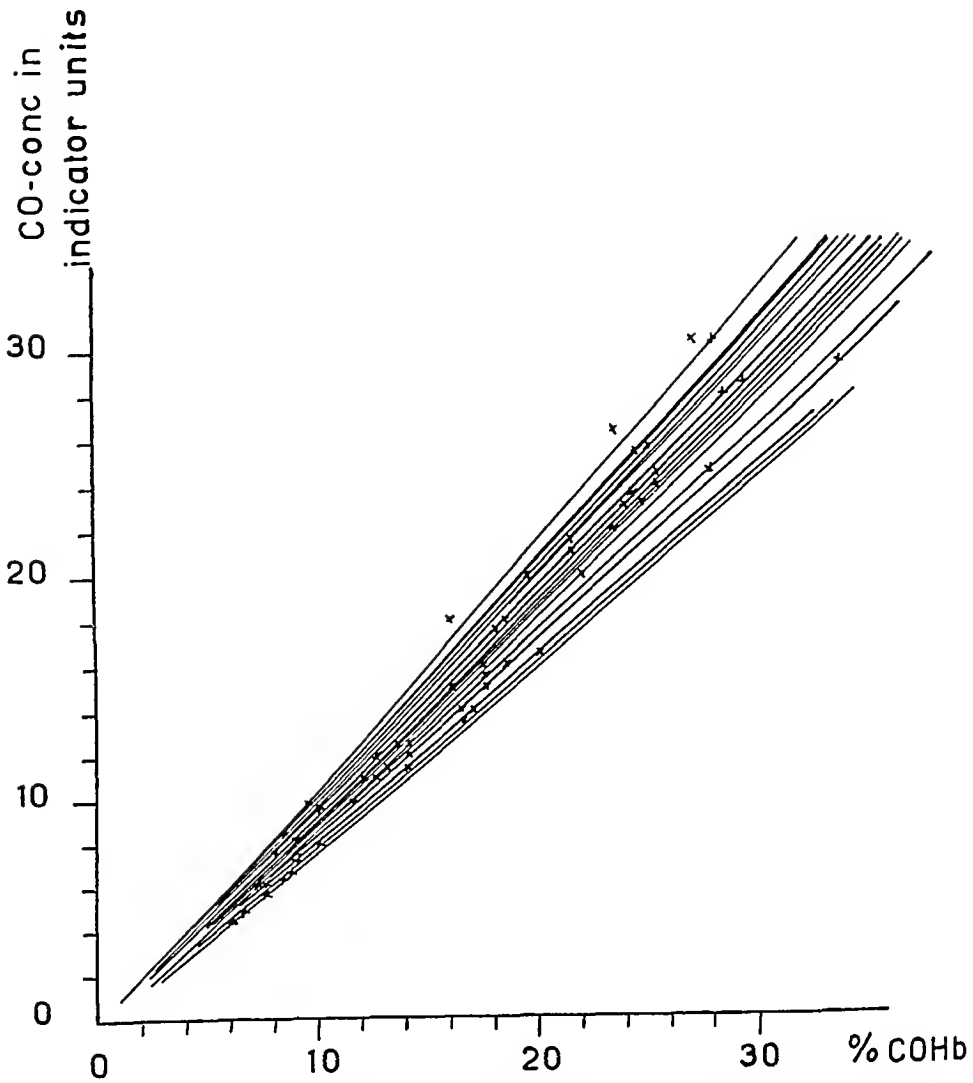

Fig. 5. The measured and calculated COHb values for 23 different individuals. For full description see text.

From these investigations it would seem that the alveolar air method with determinations on the same individual gives a difference between the single determinations of about ± 3 %, and by comparing different individuals of less than ± 10 % of a mean value. This degree of accuracy renders the method particularly suitable for low COHb values. In such cases more exact determinations should be possible than with direct COHb methods. For practical purposes the accuracy is generally quite sufficient, even at higher COHb-concentrations.

Discussion.

In the first place the method was intended to be a simple, practical and easily applied one for determining the COHb, and a quick sign of carbon monoxide poisoning. Its accuracy is so high, and possible of still further improvement by using oxygen and carbon dioxide absorption (see SJÖSTRAND, 1948), that it is also suitable for scientific analysis of CO absorption and elimination. It has already been used for the latter purpose by GULLBERG and SVENSSON (1947).

The ease with which samples may be collected and analysed, and its adaptability to use in combination with air analysis, makes this method particularly suitable for determining the presence of CO and preventing the risk of CO poisoning at works, where CO is liberated. Already, its usefulness has been demonstrated by investigations of the following nature, carbon monoxide risk in mines (BERGDAL, BERGGREN and ÖHMAN, 1945), in gas works (FREDRIKSSON, 1945), as well as in routine investigations by the Swedish industrial safety departments.

Summary.

A method for the determination of the COHb-concentration in the blood by using the analysis of the CO-concentration in the alveolar air is described. An analytical apparatus for the measurement of low concentrations such as 0.0005 % CO in the air, was constructed for the purpose and a short description is given. The alveolar air was obtained by collecting either the exhaled air and rebreathing this 10 times, or by expiring the nitrogen from the lungs and breathing pure oxygen together with the absorption of carbon dioxide until equilibrium is reached between the air in the sample and in the lungs. The first and simpler method has getting alveolar air samples for an error of about $\pm$ 3 %. The latter method is even more accurate. A comparison of the accuracy of the method on different individuals shows a calculated error below $\pm$ 10 % of the value. The usefulness of the method is discussed.

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A Method for the Determination of the Total Haemoglobin Content of the Body.

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Up till now it has not been possible to obtain an absolute value of the degree of haemorrhage or haemoglobin deficiency in anemia or of the regeneration of the haemoglobin under these conditions. The relative haemoglobin values do not give a reliable estimation of the body's total haemoglobin content, partly because the compensatory increase of plasma does not occur parallel to the loss of blood cells, and partly because the total volume of blood changes under these conditions. It is therefore desirable, both in clinical and scientific estimations of these conditions, to be able to supplement the relative haemoglobin value with the absolute value for the haemoglobin content of the body.

The methods previously used for the estimation of blood volume are not suitable for this purpose. They may be divided into such methods where the quantity of plasma is determined and the volume of blood cells and the total amount of blood are calculated from the haematocrit value; and those where the total amount of haemoglobin or blood cell volume is determined and the total blood volume is calculated from the haematocrit value. Methods for the estimation of the plasma volume are not quite reliable; the substances used disappear too quickly from the vascular system and the calculation of the blood cell volume from the haematocrit value is not accurate, because a specimen of venous blood from,

for instance the cubital vein, is not representative of the blood as a whole (SMITH, ARNOLD and WHIPPLE 1921, FÅHRÆUS, 1928).

Of the blood cell methods the oldest is the carbon monoxide one, introduced by GRÉHANT and QUINQUAUD 1882 with dogs and by HALDANE and SMITH, 1899, with human beings. In this method a known quantity of carbon monoxide is administered, thereafter the COHb concentration is determined. It has been used in a great number of scientific experiments, although the original method has been modified, particularly with respect to the technique for the determination of the carboxy-haemoglobin and to the time interval between the administration of carbon monoxide and the taking of the blood test. The method has given many varying values, in all probability because the methods for the COHb determinations are neither dependable nor directly comparable, and also because it has not been generally recognized that practically every individual has a certain concentration of COHb in the blood, which varies to a large degree with different living conditions and with the smoking of tobacco.

The earliest experiments with this method suffered also from the fact that the blood tests for the determination of the COHb were made before complete mixing was attained. After the importance of this had been recognized, the values obtained by the CO method have given throughout too high values in comparison to other methods. This may be due in part to the fact that the quantity of carbon monoxide taken up by the blood, is not adequately determined, and partly to the methods used for the determination of the carboxyhaemoglobin not being quite reliable. Another source of error in the CO method is that a part of the carbon monoxide is taken up by the myoglobin; this quantity is unknown and can be expected to vary in different individuals as well as under different physiological conditions. The CO method, in the form employed up to now, is consequently coupled with too many sources of errors to be suitable as a clinical method for the determination of total haemoglobin; and besides, relatively large quantities of carbon monoxide must be administered in order to make the measurements of the COHb comparably reliable, whereby the oxygen capacity of the blood is reduced to such an extent that complications may arise in cases of haemorrhage and anemia.

Recently, methods which have been developed by HAHN and HEVESY (1940), HEVESY and ZEHRAHN (1942), HAHN and as-

sociates (1941, 1942) for the determination of the volume of blood cells, have been used in scientific experiments. In these the blood cells in a blood specimen are labelled with radioactive phosphorus or iron and then reinjected; the radioactivity in a sample of the blood is then determined.

Another method for the determination of blood volume using labelled blood cells has been used by HEDENSTEDT (1947) namely with the help of blood transfusions of elliptocytes taken from individuals with a preponderance of elliptically shaped blood cells. In this way HEDENSTEDT also made blood volume estimations using doubly labelled blood cells *i. e.* elliptocytes with radioactive phosphorus, and found in some children that these two methods did not give the same values. This he explained by the fact that radioactive phosphorus escapes from the blood cells more quickly than was earlier assumed.

On a large number of individuals, principally children, he also found a 30 % lower value of the blood volume with the elliptocyte methods than had been obtained with the radioactive labelled blood cells by HEVESY et al. (1944). However, none of these methods with labelled blood cells is suitable for the clinical determination of total haemoglobin, since they are relatively complicated, requiring certain preparations and besides, repeated measurements are not possible.

In the following pages a method is described which is a modification of the carbon monoxide method, and which has been shown to give more accurate determinations than the methods previously used for the estimation of the blood corpuscle weight. At the same time it can be used on individuals suffering from haemorrhage or shock without causing complications. Since the method is relatively simple to employ, it is suitable for clinical examinations as well.

Principal Considerations.

Fundamentally, the new method consists in the administration of a known quantity of carbon monoxide and the estimation of the COHb concentration in the blood by the analysis of the CO concentration of the alveolar air according to the technique previously published (SJÖSTRAND, 1944 and 1948).

The individual, after breathing 100 % oxygen in order to clear the lungs of the greater part of the nitrogen, is allowed to breathe

through a system of tubes filled with 100 % oxygen and supplied with a carbon dioxide filter and a rubber bag. He continues to breathe in this system until equilibrium is reached between the COHb concentration in the blood and the CO concentration of the system, inclusive of the lungs. The CO concentration of the system is determined before and after the administration of carbon monoxide, and from the difference between these two values the total haemoglobin is calculated. The method can determine very low COHb concentrations (0.02 %), therefore only small amounts of carbon monoxide need to be administered (10—30 ml).

This method is based on the assumption that the COHb concentration remains constant for fixed O_2 and CO pressures in the alveoli, in accordance with the formula $\frac{COHb}{O_2Hb} = \frac{MpCO}{pO_2}$, and that M does not vary to any marked degree in different individuals. This has been discussed in an earlier paper (SJÖSTRAND, 1948).

Apparatus and Technique.

Determination of the CO concentration is performed with the apparatus previously described (SJÖSTRAND, 1948). By calibrating between every, or every second calculation, and by avoiding changes in temperature in the laboratory room the accuracy of the CO determinations was kept somewhat higher than that previously noted, as a rule within ± 1.5 %.

The apparatus in Fig. 1 is used for getting the samples of alveolar air. It consists of an oxygen container and two 7 litre rubber bags, which by means of a special stop-cock can be made to change places and alternately be connected to the rest of the system. The latter consists of connecting pieces, rubber tubes, respiration valve with mouth piece, carbon dioxide filter and a three-way cock whereby the system can be entirely closed or opened to the atmospheric air through one tube. In addition there is a special connection supplied with a rubber membrane through which carbon monoxide can be administered. The respiration valve and the diameter of the rubber tubes, the smallest $2^1/_2$ cm., were chosen in order to reduce the resistance in the system to a minimum.

The carbon monoxide mixture holding an CO_4 content of 92 % was supplied by AB Kebo in containers. It was injected into the system through the rubber membrane, with a calibrated 20 ml. Record syringe sealed with paraffin oil.

In making the determinations, oxygen is first passed through the system, finally filling the bags. Afterwards, the mouthpiece is placed on the person, who then for 4 minutes breathed in from one of the rubber bags continuously supplied with oxygen, and out through the

opening in the three-way stop-cock to the free air. The system is then closed by turning the three-way stop-cock, and the rubber bag which was now connected to the system is filled with oxygen; thereafter the supply of oxygen is stopped. After 7 minutes the bag is refilled with oxygen. After 15 minutes the bags are exchanged and carbon monoxide administered. After another 15 minutes the patient is asked to breathe out easily; the rubber bag is closed and the test completed.

Sometimes 2 specimens were taken before the administration of carbon monoxide and sometimes even more after. In this case several rubber bags were fitted to the system.

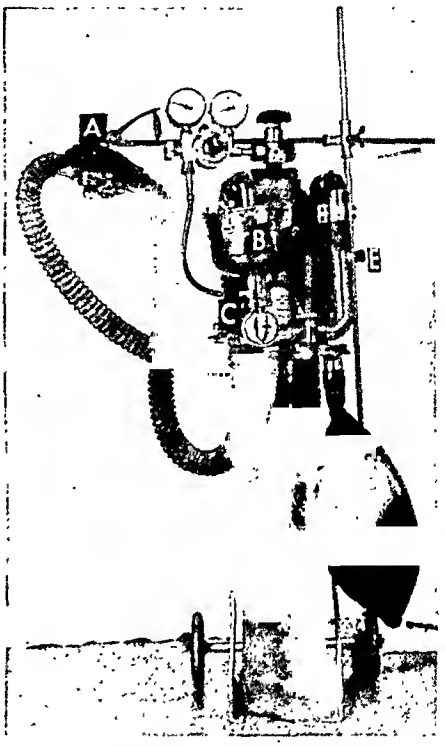

Fig. 1. Apparatus for the determination of total haemoglobin. A, respiratory valve with mouth piece. B, CO_2-filter. C, three-way cock with opening outwards. E, opening covered with membrane for the injection of CO.

Calculation of the Total Haemoglobin.

The total carbon monoxide capacity of the blood is obtained from the formula $K = \frac{V \times 100}{c}$, where V is the quantity of carbon monoxide absorbed by the blood (volume at 760 mm. Hg and 0° C), and c is the COHb concentration (in vol. %). When K is multiplied by 0.736, the amount of haemoglobin in grams is found (Hb total). The volume (V) is the amount of carbon monoxide administered less that amount present in the lungs and in the outer system. The latter can be calculated from the measured CO concentration and the gas volume in the system, according to $V = A - \frac{a}{100}(P + S)$, where A = the carbon monoxide administered, a the measured CO concentration, P = the lung volume after a normal expiration (at the completion of the test) and S = the volume of the system.

c can be calculated from the determined value for the CO concentration in the system (a), the alveolar O_2 pressure (p), from a known relationship between alveolar CO concentration (b) and COHb (c_1) at a fixed alveolar O_2 pressure (p_1).

Consequently $c = \frac{a \times c_1 \times p_1}{b \times p}$

The estimation of the total haemoglobin can thus be made according to the equation

$$\text{Hb total} = \frac{[A - \frac{a}{100}(P + S)] \times [0.736 \times 100 \times b \times p]}{a \times c_1 \times p_1}$$

A is measured and a is found by determination, P can be calculated and consists of the residual air plus a great part of the reserve air volume, S is the volume of the apparatus inclusive of the rubber bag, at the end of the determination and can easily be determined, b can be fixed at 0.01 % whereby $c_1 = 15$ % and $p_1 = 100$, p is calculated from the O_2 concentration of the system, the alveolar CO_2 pressure and the water vapor pressure which can be assumed to be the same as when breathing ordinary air. In order to simplify the calculation, P can be approximated and in the following experiments was set at 3,000 ml. By so doing, variations in the lung volume can naturally influence the accuracy of the determinations. However, a variation of about 1,000 ml. usually gives an error of below 1 % only. In cases of more marked emphysema and in those with low total haemoglobin value a correction should be made. In the technique used here, the O_2 concentration has varied between 90 and 95 %; and 610 mm. Hg has been calculated as the average alveolar O_2 pressure at an atmospheric pressure of 760 mm. Hg. This value is included in the equation in relation to the CO pressure and therefore no correction is necessary for variations in the atmospheric pressure, subsequently $\frac{b \times p \times 0.736}{c_1 \times p_1}$ may be replaced by a constant.

With this approximation, the variations in the O_2 concentration of the system give rise to a possible error, which in a few cases may approach 3 %. Under exceptional conditions such as lung emphysema, this error may be even greater. This can be counteracted by allowing the patient to breathe oxygen for a longer time before closing the system. A systematic error in the calculations may lie in the use of the value 15 as the value for COHb, with a CO concentration of 0.01 % in the breathing air and an O_2 pressure of 100 mm. Hg. This figure, judging from earlier experiments, should be fairly near the true one (see SJÖSTRAND, 1948). Individual variations in this value may constitute another source of error, which, however, should not be large under normal conditions considering the determinations made by SENDROY, LIU and VAN SLYKE (1929).

Errors of the Method.

The method requires that the testing time, 15 minutes, is sufficient for a balance to be reached between the air in the bag, the alveolar air, and the CO tension in the blood. Within these 15 minutes the COHb concentration must become the same throughout the entire vascular system. An essential part of the body's total blood content

during rest has been assumed to be retained in the "depot organs", of which some, the so called "true depot organs", partially segregate their contents from the rest of the circulating blood. In order to test the extent to which equilibrium is actually reached between the air sample in the rubber bag and the blood during 15 minutes, repeated determinations of the alveolar CO concentration were made after the administration of CO, as well as experiments to release the blood from any possible "true depot organs".

Repeated Determinations of the Alveolar CO Concentration Following Administration of a Fixed Quantity of CO.

Several investigators have found with different methods, that the conditions for mixing the blood in the vascular system in human beings are good enough to give almost a complete mixing within 5—8 minutes. This fact can easily be demonstrated with the administration of carbon monoxide through a rebreathing system of small size and determining the alveolar CO concentration with the method earlier described (SJÖSTRAND 1948). The result of such an experiment is shown in the figure 2. There are, consequently, sufficient possibilities in this respect for reaching equilibrium during the testing time here used.

In a large number of experiments several 15 minute samples have been taken after the usual test. If the values obtained are plotted with time along the horizontal axis, diagrams of the type illustrated in Figs. 4, 5 and 6 are found. Between the first and second value after administration of CO the difference is greater than between the subsequent values, which as a rule show a steady decrease. This decrease is for the most part caused by the removal with each specimen of a certain quantity of CO from the system. In addition, it is quite possible that an extremely small quantity of the CO is eliminated in another way or is taken up somewhere in the body.

The more marked decrease between the first and second test, which reached about 5 % above the expected decrease, may be caused by a small quantity of blood not having taken up the CO to the same concentration as the rest of the blood during the first 15 minutes. Another explanation is, however, that some of the CO unites with the myoglobin and equilibrium between the CO concentration of the blood and the myoglobin is not reached in 15 minutes, an assumption which will be discussed later on. One fact among others, which supports this, is that the procentual

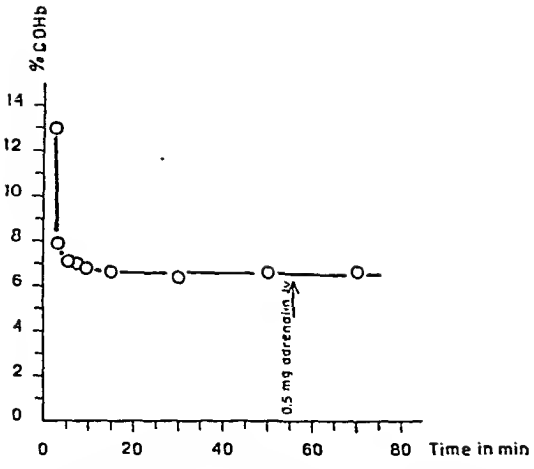

Fig. 2. The alveolar CO-concentration after administration of CO by the lungs. For description see the text.

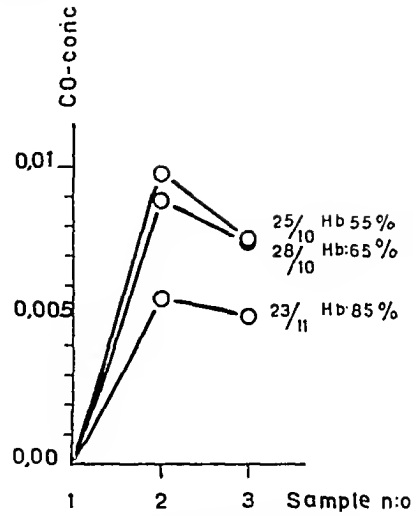

Fig. 3. The relationship between 15 and 30 minutes samples on one and the same individual during Hb-regeneration after acute bleeding.

difference between the second and the third samples is the same in normal individuals as in out-bled patients with only 50 % relative haemoglobin, and even in the same individual at low Hb values, as well as at normal (see fig. 3). It can, of course, be expected that in anemia the "blood depots" are emptied.

These observations indicate consequently that equilibrium between the CO in the lungs and the blood is reached after 15 minutes, but that a small amount CO after this time is taken up somewhere else, probably in the myoglobin.

In a large number of experiments both 15 and 30 minutes specimens have been used for the calculation of the total Hb. In these the 15 minute values have shown the least individual variations and the best correlation with body weight. It has therefore been considered most accurate to use the 15 minute values for normal routine determinations. It should be pointed out, however, that the conditions for mixing may be expected to be decidedly inferior in, for example, peripheral circulatory insufficiencies than in normal circulatory conditions. In such circumstances it is advisable to include even the 30 minute value; and if this is much lower than the 15 minute one, to use the former after correction for the CO quantity eliminated with the 15 minute sample and the difference of 5 % normally appearing between the 15 and 30 minutes values.

Experiments Concerning the Emptying of Possible True Blood Depots.

In order to empty the blood from possible blood depots, which had not been mixed with the circulating blood during the mixing time, repeated determinations of the alveolar CO concentration were made and the effect of adrenalin, hard work and increased skin temperature on this was studied.

Two individuals were given 0.25 and 0.30 mg. adrenalin respectively by slow intravenous injection, which caused a strong reaction of the blood vessels. The curves in Fig. 4 show that there was no further dilution of the blood, which was to be expected if appreciable quantities of blood had been stored in true blood depots.

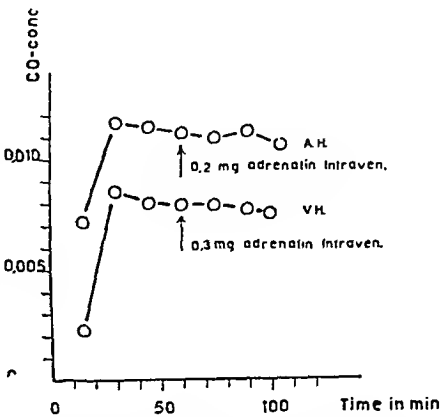

Fig. 4. Alveolar CO concentration in two individuals after administration of CO. At ↑ intravenous injection of adrenalin.

Three females during a similar test performed relatively heavy work on a bicycle-ergometer (600 kg.m/min.) for 15 minutes. The graphs of these experiments are to be found in Fig. 5 together with those during rest. As can be seen, the alveolar CO concentration shows a lower value than expected during heavy work. This does not depend, however, on the emptying of blood from depots, since the CO value after a subsequent 15 minutes of rest shows dilution to the expected, or even somewhat higher value. That this condition is not dependent on a lowered alveolar CO_2 pressure due to hyperventilation, has been evidenced by direct measurements which showed to the contrary that the CO_2 concentration in the system was increased.

A reasonable explanation for this decrease in the alveolar CO concentration during heavy work, seems to be that there is a greater absorption of the carbon monoxide by the myoglobin than during rest. ASMUSSEN (1942) tested this assumption but did not find that this was so. That can be expected, however, since during work the oxygen tension and the temperature of the muscles increase and the pH very probably falls.

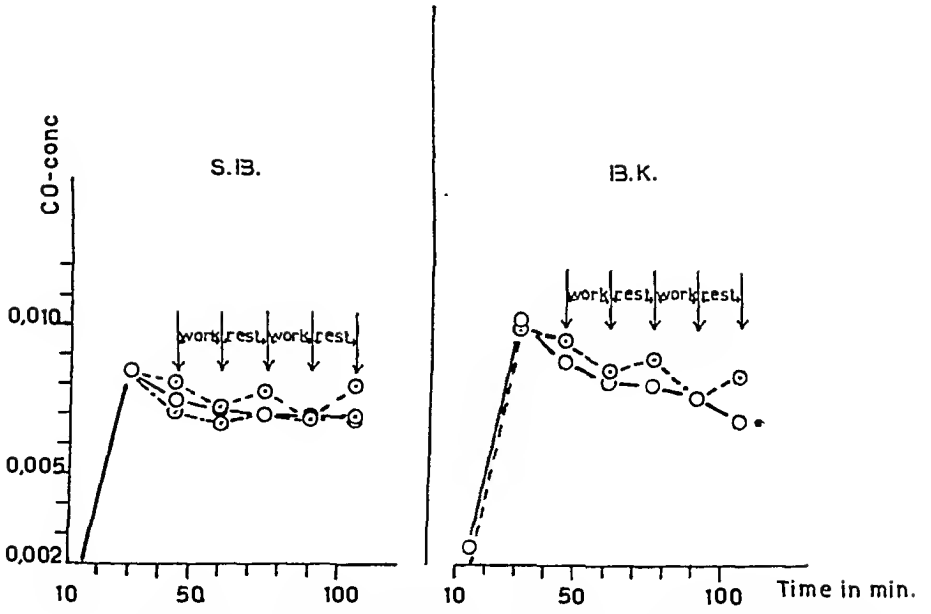

Fig. 5. Alveolar CO-concentration after administration of CO also before (continuous line) and after (dashed line) work on a bicycle-ergometer.

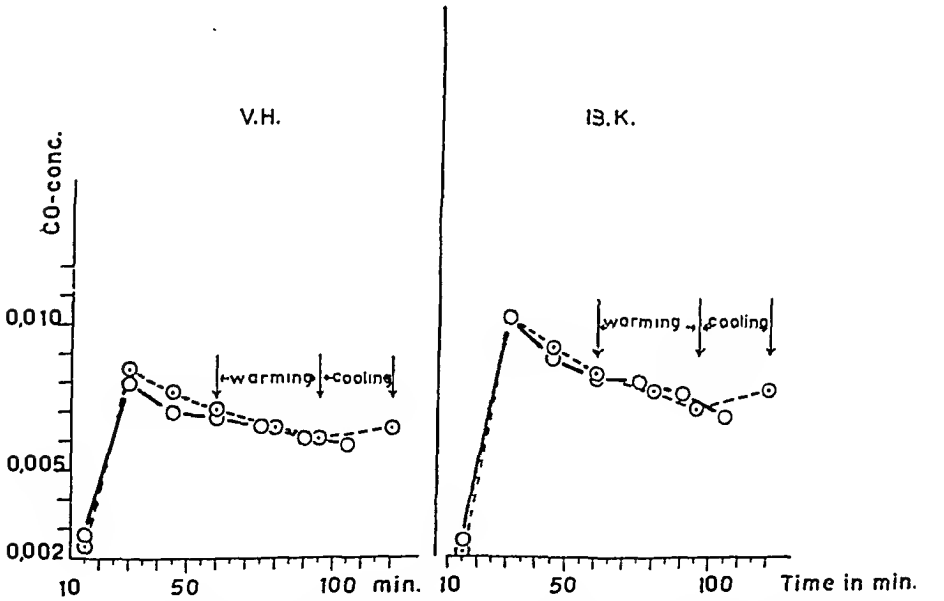

Fig. 6. Alveolar CO concentration in two individuals after the administration of CO. The continuous line experiment in ordinary room temperature. Dashed line before, during and after heating in a heat chamber.

As THEORELL (1934) showed, the dissociation curve of oxymyoglobin is influenced much less by changes in temperature and pH than that of oxyhaemoglobin, which should lead to a change even

in the quantitative relationship between CO myoglobin and CO haemoglobin in the muscles at constant O_2 pressure. It is of course also possible that the dissociation constant for CO haemoglobin varies in some way, at present unknown, during heavy work. Even if the observed variations of the alveolar CO concentration are relatively small, the maximum being 6 % in the present experiments, they should be considered, since the observations show that under certain conditions the CO method can include greater errors than under normal conditions.

According to the fundamental experiments by J. BARCROFT (1926) on the variations in the amount of circulating blood and in which he used the CO method, heat stimulus produces a very decided increase in this amount. These observations led to a search for true blood depots in the body and to the rediscovery of the part played in this respect by the spleen. It was therefore decided to test the effect of a strong heat stimulus with the present method.

Three individuals were exposed to heat in a heat chamber having an opening for the head and the other conditions being the same as in the experiments with adrenalin and heavy work. After the administration of carbon monoxide and the collection of three 15 minute samples the individual was placed in the heat chamber at a temperature of 45—50° C, thereafter two 15 minute samples were taken. The chamber was cooled and a new 15 minute specimen collected. In Fig. 6 the results are shown from two of these experiments.

It is apparent that during heating of the body the alveolar CO concentration is decreased somewhat more than was calculated, only to rise again to the same or slightly higher value than was to be expected. This phenomenon was just as clearly demonstrated in the experiment on a third individual. The decrease in the alveolar CO concentration in connection with heating was in this case 4—5 % more than estimated.

Just as in the case of the variations during heavy work, there can be no question of an emptying of blood depots. Possibly the condition stated above plays some part in this effect, namely that the dissociation constant for oxymyoglobin varies to a lesser degree than for haemoglobin, during changes in temperature. Even if the total body temperature is only insignificantly altered by heating in this manner, the average temperature of the muscles of the extremities is raised appreciably.

These experiments with heating and exercise have shown that

with the CO method an apparent increase in the amount of haemoglobin can occur, which is not related to an emptying of blood from depot organs. The observations made by BARCROFT on the effect of heat on the volume of blood, and which he interpreted as blood depot effects, seem to have another explanation.

It should perhaps be pointed out in this connection, that although a comparably good mixing of the administered CO with the blood is established within 15 minutes, this must not be taken as an indication that human beings lack blood depot organs. The fact shows only that existing blood depots in humans do not function as true depots, *i. e.* hold the blood entirely separate from the circulating blood. Blood deposited in the lungs whether it is circulating or not (SJÖSTRAND 1935, 1940) must be assumed to absorb CO to an adequate concentration with respect to the CO pressure of the alveolar air. It seems apparent also from the observations of HEDENSTEDT (1947) with transfusions of elliptocytes, that by this method it takes a somewhat longer time to reach a complete mixture.

The Uptake of Carbon Monoxide by the Myoglobin.

All methods by which the total haemoglobin is calculated by the help of CO are subject to the error caused by the absorption of a quantity of the CO by the myoglobin. This quantity may vary in different individuals and very probably even in one and the same individual, under different conditions, as has already been discussed. This signifies, therefore, that it can not be only a systematic error.

In table 1 some factors influencing the uptake of CO in the myoglobin are stated and some probable data are mentioned about the O_2 and CO pressure in the muscle capillaries and muscle tissue during the test time here used. It is, however, not possible to give adequate figures about the relative affinities of the haemoglobin and myoglobin for CO, valid for the low pressures in question here, because the respective dissociation curves are not of the same form. The comparatively slow circulation through the muscles, the low CO pressure gradient, and possibly also the difference in affinity for CO, will probably reduce the amount of CO taken up in the myoglobin during the test time to a great extent. The error due to the uptake of CO in the myoglobin will consequently be greater, the longer the time taken for the test.

Table 1.

Factors influencing the CO uptake in the myoglobin.

A. The dissociation of COHb and diffusion of CO to the myoglobin.

1) The "affinity" $= \frac{pO_2}{pCO}$ by half saturation $= \frac{250}{1}\left(\frac{O_2Hb}{COHb} = \frac{1}{1}\right)$

2) The velocity of COHb-dissociation = 6.9 sec. for half dissociation (ROUGHTON 1945).

3) The blood flow through the musculature, *i. e.* = $\frac{\text{the volume of blood flowing through the muscle capillaries p. min.}}{\text{the total blood volume,}}$

4) The diffusion time for CO; haemoglobin — myoglobin (the diffusion distance, diffusion constants, tension gradient).

* * *

5) $\frac{pO_2}{pCO}$ in the muscle capillaries can be calculated approximately =

$$= \frac{30}{0.0025} \text{ mm Hg} = \frac{12{,}000}{1}\left(\frac{O_2Hb}{COHb} = \frac{50}{1}\right) ?$$

B. The CO-uptake in the myoglobin.

6) The "affinity" $= \frac{pO_2}{pCO}$ at half saturation $= \frac{13.9}{1}\left(\frac{O_2Mb}{COMb} = \frac{1}{1}\right)$ (THEORELL, 1934, horse Mb.)

7) "Co combination", millimol $^{-1}$ sec. $^{-1}$ = 300 (Hb = 130) (ref. MILLICAN, 1939).

* * *

8) $\frac{pO_2}{pCO}$ in muscle tissue can approximately be calculated =

$$\frac{15}{0.0025} \text{ mm Hg} = \frac{6{,}000}{1}\left(\frac{O_2Mb}{COMb} = \frac{430}{1}\right) ?$$

9) The total CO capacity of the myoglobin = 20—25 % of that of the blood.

It seems also reasonable that this is the cause of the difference between the 15 and 30 minute samples, as already discussed.

Accuracy of the Method.

With the intention of testing the accuracy of the method on the same individual, repeated determinations were made on two persons during $6^1/_2$ and 8 months respectively. In Table 2 the

Table 2.

Mean, standard error of the Mean and standard deviations by repeated determinations of the total haemoglobin on two individuals.

Test-person	Observation period	Number of determinations	Total Hb Mean	Standard error of the Mean	Standard deviation in per cent
B. K.	$^{17}/_{4}$—$^{13}/_{12}$	15	435	± 3.58	3.2
V. H.	$^{5}/_{5}$—$^{13}/_{11}$	15	630	± 6.5	4.0

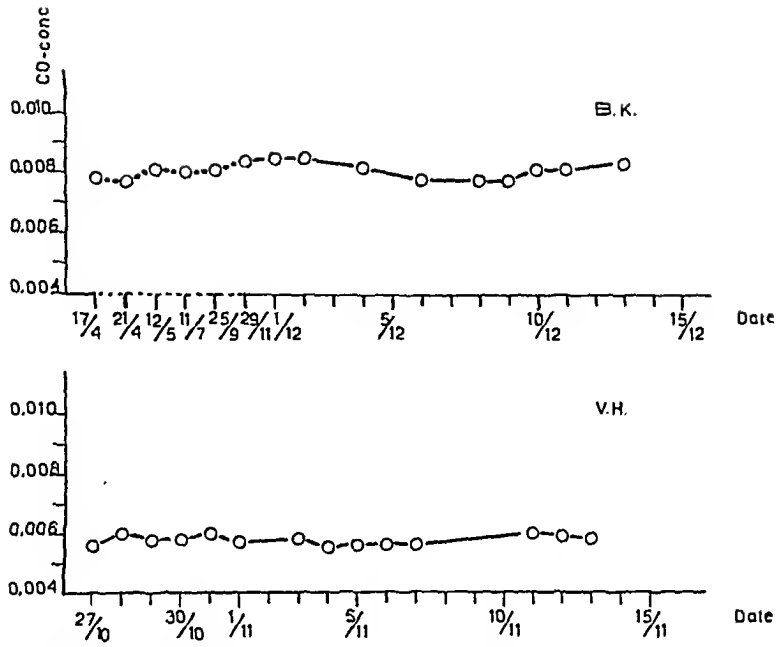

Fig. 7. Repeated determinations of the total haemoglobin in two individuals.

average values are given, and in Fig. 7 the values have been plotted on a graph. As appears from the table, the standard deviations in this series are 3.2 % and 4 %. It is possible that a part of the differences is caused by real fluctuations in the amount of haemoglobin. In one individual (B. K.), the relative Hb values also varied between 75 % and 85 % during the observation period.

The accuracy of the method used on the same person has also been apparent in the determinations made before and after bloodletting and transfusion. The results have been collected in Table 3. From this it appears that changes in the Hb, calculated from the total Hb determinations, correspond to the blood quantities measured directly to within $\pm$ 12.5 g. Hb (= 83 ml. blood). The greatest error was 2 % of the value.

The accuracy of the method used on different individuals is more difficult to fix, because comparisons cannot be made with still more accurate methods, and the total haemoglobin cannot be calculated exactly from the body weight or from the body surface. It can be expected, however, that some of the previously named sources of error may be of some importance. Determinations of the total Hb on 117 males with relative Hb values varying between 85 and 105 %, and who showed no signs of blood or

Table 3.

Determination of total Hb before and after bleeding and transfusion.

No.	Test-person	Date	Body weight in kg.	Total Hb before bl. in g.	Amt. drawn		Amt. transf.		Determined diff. in gram Hb.	A—B
					ml.	g. Hb A	ml.	g. Hb A		
1	A. S.		76	787	290	44			51.5	— 7.5
2	T. A.		76	877	300	45			32.5	+ 12.5
3[1]	G. C.		77	826	290	44			55	— 11
4[1]	U. K.		84	819	315	47.5			47	+ 0.5
5[1]	G. K.		69	771	300	45			52	— 7
6	L. B.	$^{27}/_{10}$		[2]			250	37.5	29	+ 8.5
7	»	$^{31}/_{10}$		[2]			500	75	64.5	+ 10.5
8	»	$^{7}/_{11}$		[2]			500	75	83	— 8

[1] Double quantity of CO = 30 ml. uncorrected volume.
[2] See Fig. 8.

heart diseases, gave an average of 1.124 % ± 0.0096 per cent Hb of body weight with a standard deviation of ± 0.104 (= 9.2 %) and 425 ± 3.6 g. per square meter body surface, with a standard deviation of 38.8 g. (= 9.1 %). On 69 women, without any signs of blood or heart diseases, the corresponding values were 0.86 ± 0.0229 per cent, ± 0.102 (11.9 %), 321 ± 4.5 g. and 37.8 (11.7 %) respectively.

This spread in the standard deviations is in all probability only partly due to errors in the method, and mainly to individual variations. It can be assumed that the blood volume varies individually in relation to the body weight or surface, just as the weight of other organs does, *e. g.* the heart and liver. The difference between the sexes is about the same for example, as that between the heart volumes, determined by the X-ray method.

In order to lessen the error in determinations it is important in relatively high initial values of COHb, as in smokers, to decrease the COHb concentration before the determinations, since the error of the CO meter is directly proportional to the measured CO concentration. With high initial values, two determinations should be made prior to the administration of CO, in order to correct for the CO quantity eliminated at each separate test. This is not necessary with low COHb concentrations, even if it does have some small effect. Accidental errors, such as the mouth piece not being properly applied to the patient, can of course influence the results to a marked degree. When this is suspected, double

tests should always be made. The accuracy can be further increased if tests are made using larger quantities of CO (30—40 ml.), and if care is taken to clear the nitrogen entirely from the lungs before taking samples. For routine use, however, the procedure described above should be sufficiently accurate.

A Comparison Between Values of the Blood Corpuscles Weight Calculated from the Total Hb-values Obtained with the Present Method, and Those Obtained with Some other Methods.

If a calculation of the weight of the blood cells is made from the weight of the total haemoglobin in relation to body weight and a Hb concentration in the red blood cells of 35 %, a comparison can be made between the values obtained by the method described here and those obtained by some other methods as is shown in table 4. From this it can be seen that the figures for men, obtained with the present method, lie 16 % above the values obtained with the elliptocyte method and 12 % below the values received with the radioactive phosphorus method. The values for women are below those given in the mentioned investigations, in which no separation between the sexes is made, thus making a direct comparison impossible. According to HEDENSTEDT's investigations reported in the introduction, the method used by HEVESY and his associates gives too high values, because the radioactive phosphorus leaves the blood more rapidly

Table 4.

Total weight of red corpuscles in per cent of the body weight determined with different methods.

Method	No. of cases	Weight border values in kg.	Sex.	M ± ε (M)	σ	σ in % of M.
Radioactive P (HEVESY et al. 1944)....	21	54—72		3.60 ± 0.09	0.417	11.6
Carbon monoxide (HEVESY et al. 1944)..	19	54—72		4.42 ± 0.15	0.653	14.8
Elliptocytes (HEDENSTEDT, 1947)	19	5—78		2.70 ± 0.11	0.47	17.5
Carbon monoxide modif. SJÖSTRAND....	117	53—98	Men	3.22 ± 0.027	0.29	9.1
Carbon monoxide modif. SJÖSTRAND....	69	40—88	Women	2.43 ± 0.034	0.30	12.3

than was at first believed. It may be pointed out that HEDENSTEDT's value for the blood corpuscles' weight is an average of relatively few cases, practically all children. It is probable that the blood volume and total haemoglobin content is not correlated only to body weight, but also to the body height (or the body surface), and as found in the material observed in the present investigation, there is a comparably great difference between sexes. This would mean that HEDENSTEDT's value is not directly applicable to adults.

The great difference between the CO method and the radioactive phosphorus method found by HEVESY et al., is probably due to some errors in their calculations. Their values, obtained with the CO method, do not correspond to the same values of other investigators (see for example ASMUSSEN 1942). From the description of the technique it looks as if HEVESY et al. calculate the blood volume on the assumption that the whole amount of CO in the spirometer is taken up in the blood during the test period, an erroneous assumption which will give at least 10 % too high a value when the spirometer is filled with oxygen.

It seems to be apparent from this comparison with some other methods that the present method does not have greater systematic and variation errors, even including that of the CO uniting with the myoglobin, than the methods previously considered to be the most dependable for the estimation of the weight of blood cells.

Determination of the Total-Hb after Haemorrhage.

Fig. 8 is a diagram of the total haemoglobin content and the relative haemoglobin values in a patient admitted to hospital with severe bleeding from a duodenal ulcer.

The diagram shows the effect of three blood transfusions (included in Table 3), and the average daily increase in the total haemoglobin value after the cessation of bleeding. Excluding the effect of the blood transfusions, the daily formation of haemoglobin can be estimated about 4 g. In another similar case the formation of haemoglobin was 5.2 g. per day. In Fig. 9 the relative and absolute Hb values are plotted in a coordinate system, which demonstrates that there is no direct relationship between these values; the relative values are comparatively higher than the absolute. This would seem to depend on the fact that the increase

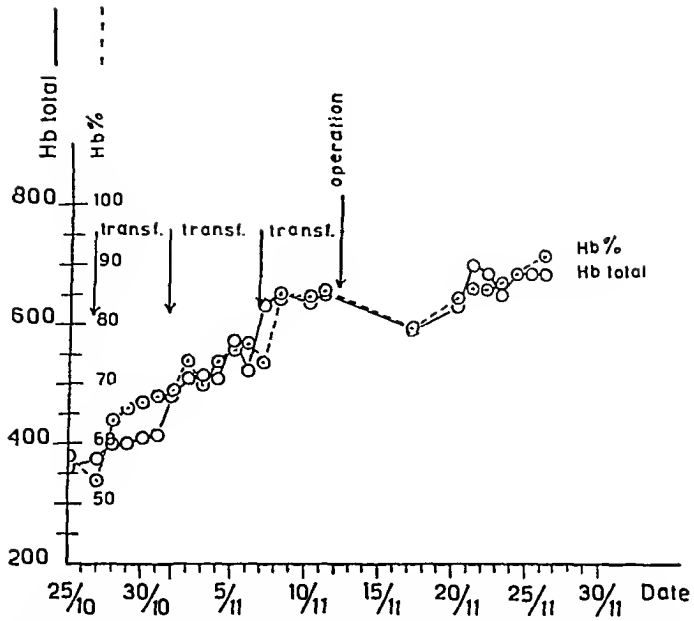

Fig. 8. The total weight and relative values of haemoglobin after haemorrhage from ulcus duodeni.

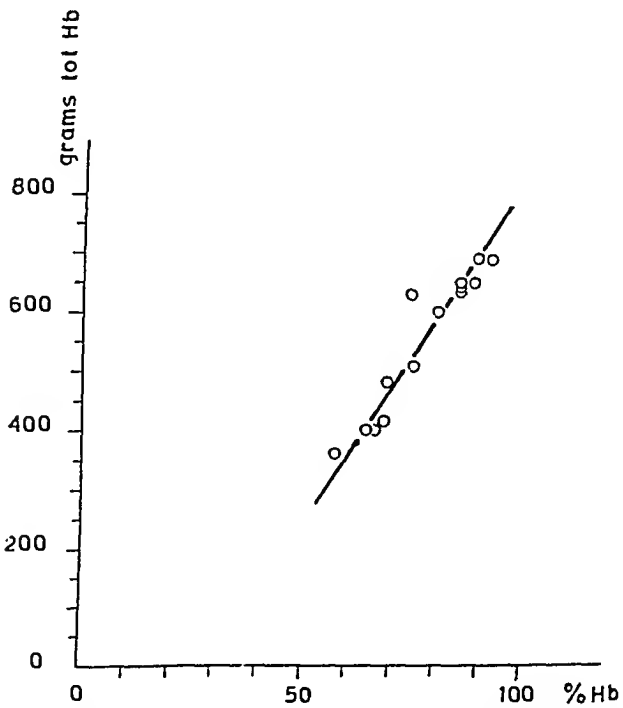

Fig. 9. The relation between the total weight and the relative values of haemoglobin during regeneration following haemorrhage in the same case as in Fig. 8.

in plasma does not wholly compensate for the loss of blood during haemorrhage. It is obvious that determinations of the total haemoglobin, more than those of the relative haemoglobin give an opportunity to follow the formation of the blood and to measure the extent of bleeding. The estimation of the total haemoglobin also makes it possible to discover internal bleeding.

Discussion.

The present method for the determination of total haemoglobin makes it possible to determine variations in the haemoglobin content for the same individual, with much greater accuracy than was possible with earlier methods. Even in making estimations from different individuals, the error seems at least not to be greater than in the best methods formerly employed for the estimation of blood corpuscle weight, such as with radioavtive phosphorus labelled blood cells; at the same time it has the advantage of being controllable by repeated determinations. The method has one source of error in that a certain undeterminable quantity of carbon monoxide is absorbed by the myoglobin. Under certain standard conditions, such as relative rest and normal body temperature, this error generally varies with the body weight and seems to be very small.

The method allows for several tests to be made also on the same day, thereby fixing the extent of bleeding. By making daily tests, the regeneration of haemoglobin following haemorrhage or treatment of anemia can be exactly determined. It can be used on individuals suffering from severe haemorrhage and shock without causing complications by decreasing the oxygen carrying ability of the blood during the test or afterwards if the patient is allowed to breathe oxygen for a time following it. The low COHb concentrations are namely compensated by an increase in the quantity of physically dissolved oxygen. Since the method is easy to employ, the tests can be made by the usual laboratory technician and in hospitals by the laboratory nurses.

Summary.

A method for the estimation of total haemoglobin has been described, involving the determinations of the alveolar CO concentration before and after the administration of carbon monoxide.

A CO-meter previously described, is used to measure the CO concentration even a low one with great accuracy. The alveolar air sample is obtained by rebreathing in a closed system fitted with a carbon dioxide filter and filled with 90—95 % oxygen.

The accuracy of the method used on the same individual is comparable to a standard deviation of $\pm$ 3—4 % and can be improved by performing several tests. In estimations before and after bleeding and blood transfusions the accuracy was still higher. In this way the total haemoglobin content has been determined for 117 apparently healthy males, showing an average total haemoglobin of 1.126 % of body weight and a standard deviation of 9.2 %. The total haemoglobin calculated on the body surface was 425 g. per m^2 body area with a standard deviation of 9.1 %. On 69 women the corresponding figures were 0.86 % of body weight ($\sigma = 11.9$ %) and 321 gram ($\sigma = 11.7$ %).

In two cases of haemorrhage the total haemoglobin was determined nearly every day; in this way the quantity of haemoglobin formed per day was found to be 4 and 5.2 g. respectively. The relative Hb values showed higher figures than could be expected from the absolute values.

The method is simple to employ and can be used in the clinic with cases of haemorrhage and shock to determine the degree of bleeding and the regeneration of the blood.

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The Exchange of Sodium and Chloride Ions Across the Fibre Membrane of the Isolated Frog Sartorius.

By

HILDE LEVI and HANS H. USSING.

Received 9 June 1948.

The purpose of the present study has been to work out a method for the determination of the rate of exchange of Na- and Cl-ions between the fibres of isolated frog sartorius and the surrounding medium.

From HEPPEL's work on rats (1939) it is known that intrafibrillar Na exchanges at a fairly high rate, an approximate equilibrium between fibre Na^{24} and serum Na^{24} being obtained within one hour. The determination of the rate of exchange was possible only on rats with increased Na concentration in the fibres, a condition brought about by feeding the animals a K-free diet.

The method used in the present paper should make possible the measurement of the rate of exchange even when the intracellular Na represents only a small fraction of the total Na content of the muscle.

The principle applied is to soak the muscle in a solution containing Na^{24} until a reasonable concentration of the isotope within the fibres is reached. Then, the washing out of the active ions with Na^{24}-free Ringer is followed over a period of time and from the rate of disappearance of activity from the muscle the diffusion rate of Na in the interspaces as well as the renewal rate of fibre Na can be estimated (see below).

The peculiar ion distribution between the muscle fibres and the surrounding solution makes imperative the assumption of an active extrusion of Na^+ from the fibres (DEAN 1941, KROGH 1946, CONWAY 1947). Based partly on the experiments to be de-

scribed below one of us (USSING 1947) has pointed out that the rate at which Na^+ is found to leave the muscle fibres — as measured with the tracer method — is not necessarily equal to the rate of active extrusion. In fact, Na^+ moves out so fast that the passing out due to active extrusion, only, becomes energetically improbable. Some mechanisms which would allow an exchange of sodium ions across the cell membrane without consumption of metabolic energy are discussed in the paper mentioned.

Technique.[1]

The radioactive isotopes, Na^{24} and Cl^{38}, were prepared in the cyclotron of the Institute of Theoretical Physics, Copenhagen, by deuteron bombardment of NaCl.

The NaCl which was mounted on a copper target was dissolved in about 10 ml of water and treated with 1 g of charcoal in order to remove traces of target material. After filtration, the Cl-content of the solution was determined on a small aliquot and the solution made up to Ringer by addition of solid NaCl and stock solutions of KCl, $CaCl_2$ and $NaHCO_3$. pH was adjusted to 7.

Sartorius muscles of curarized frogs were dissected and each muscle was immediately placed in a glass containing 1 ml of the active Ringer. (Due to the short half life of Cl^{38} (36 min.) it was impossible to follow the exchange of Cl in the muscles for more than 5—8 hours after the preparation of the isotopes. In most of the experiments, therefore, only the exchange of Na^+ was studied.)

The muscles were equilibrated with active Ringer for about two hours at the same temperature at which the rate of exchange was to be measured. Then they were taken out and wiped dry with soft filter paper. One muscle of each pair was stored for later analysis while the other was placed in the apparatus shown in fig. 1.

The muscle M is suspended between small claws forming the lower ends of two stainless steel pins A and B. Both are held in position by being fitted into bores in the ebonite plate C. While A is fixed relative to C, B can be adjusted according to the length of the muscle.

A and B may also serve as electrodes for electrical stimulation. The plate C can be moved up and down, sliding on two parallel brass rods.

The muscle is placed inside a glass tube (55 mm long, 10 mm in diameter) surrounded by a water jacket. The lower end of the tube is closed by a rubber stopper D provided with a bore for a steel cannula. This cannula is led through another rubber stopper E and reaches close to the bottom of a small glass vessel F. The stopper has another bore for a glass tube connecting F with the two way stopcock G.

In the position shown in the figure a mixture of oxygen and 1 %

[1] The authors are greatly indebted to Mr. N. O. LASSEN, M.Sc. for preparing numerous samples of radioactive NaCl, and to Mrs. L. ENGBÆK, M.D. for dissecting the muscles.

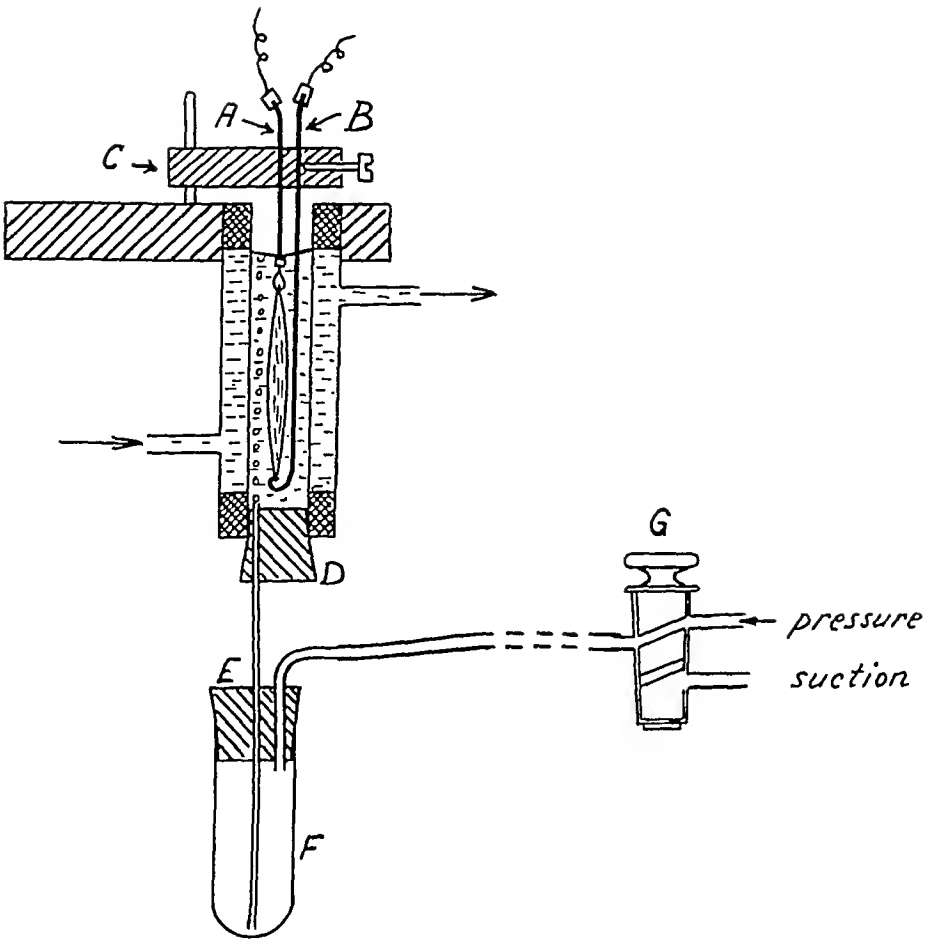

Fig. 1. Apparatus used for following the washing out of active ions from an isolated muscle, see text.

CO_2 passes through F and then through the steel cannula into the tube surrounding the muscle. The gas stream keeps the Ringer solution in the tube oxygenated and well mixed and, at the same time, the CO_2 content is so adjusted as to maintain a pH of 7.3 in the solution. When the stopcock is turned the system is connected with a suction pump and the solution is rapidly sucked down into F. This vessel can easily be removed and be replaced by a similar vessel containing 2 ml of fresh Ringer. Then the stopcock is turned again and the solution is pressed up into the space around the muscle.

This apparatus was used for the washing out of activity from the muscle. It was found that the highest accuracy was obtained when the vessel F was replaced every 2 minutes during the first 10 minute period and thereafter every 20 minutes reckoned from the beginning of the washing out process. As a rule the washing out was continued for about two hours. Then the muscle was taken out and pressed gently between sheets of soft filter paper in order to remove adhering Ringer

solution. The muscle was weighed quickly on a torsion balance and transferred to a small platinum boat or quartz test tube for ignition. The other muscle of the same pair which was not washed out was treated similarly. Ignition was performed in an electric oven at 450° C in an oxygen stream. The ash was dissolved in a given quantity of water and aliquots were taken for the Na^{24} counting and K-analysis. The potassium analysis described by SHOHL and BENNET (1928) has been slightly modified.

The potassium analysis:

Reagents: H_2PtCl_6: 1 g in 4 ml water.
Washing alcohol: 99 % alcohol, saturated with K_2PtCl_6.
Buffer solution: 4 ml 1/15 m KH_2PO_4 + 6 ml 1/15 m Na_2HPO_4 + 90 ml water.
Potassium iodide: 2 n KJ recrystallized from alcohol.

Procedure: A suitable amount (not more than 2 ml) of the solution to be analysed is measured into a pyrex centrifuge tube with pointed bottom 35 μl H_2PtCl_6-reagent is added with a Carlsberg pipette and the contents of the tube evaporated to dryness on a water bath, using a stream of hot air to promote evaporation (see SCHMIDT-NIELSEN 1945). The precipitate is freed from excess H_2PtCl_6 by repeated washings with about 300 μl washing alcohol and centrifuging for 5—10 minutes. The washing alcohol is removed by means of a fine pipette. When the alcohol appears to be colourless the precipitate is dried in a vacuum desiccator with concentrated sulphuric acid. After about 20 minutes the residual alcohol has evaporated completely. (Removal of the alcohol by heating has been abandoned, because at high temperatures the alcohol, or impurities present in the alcohol, sometimes reduce K_2PtCl_6 to metallic platinum.)

Finally, the precipitate is dissolved in 0.5 ml of boiling buffer solution (pH 6.36). After cooling, 0.25 ml KJ-reagent is added and the resulting red solution transferred with 20 ml of water to an Erlenmeyer flask. It is then ready for the colorimetric K-determination.

At optimum concentrations (about 100 γK per analysis) the error of the determinations on known solutions seldom exceeds 5 %. The results are little affected even by a sodium concentration which is 35 times higher than the potassium concentration.

When plotting the potassium content of the sample against the photometer readings we obtain a straight line, which indicates that the K_2PtJ_6-colour follows Beer's law of dilution. An example is shown in fig. 2.

The methods used to prepare samples for counting has been described elsewhere (see USSING 1948).

Calculations.

The washing out of activity from the muscle is considered essentially to consist of two different processes: taking Na as an example we have

1) the transfer of Na ions across the fibre membrane and 2) the diffusion of Na ions through the interspaces into the washing solution. We do not know how process 1) is brought about, but we make the a priori assumption that the amount of sodium ions (inactive and active)

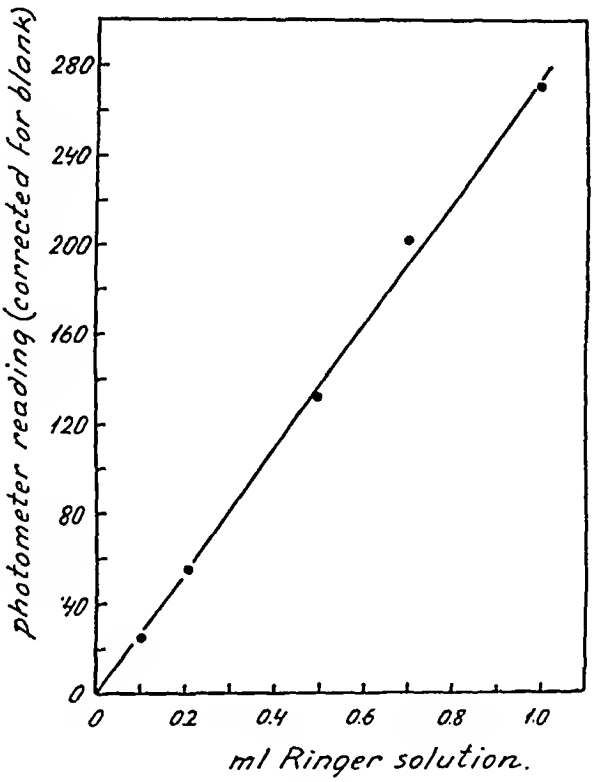

Fig. 2. The relation between K-content (given as ml Ringer solution, 4 mmolar as to K) and photometer reading, corrected for blank.

leaving the muscle fibre per unit time is proportional to the total amount of sodium present within the fibres.

Due to the diffusion out from the interspaces practically no active ions will go back into the fibres and therefore the number of active ions leaving the muscle fibres per unit time will be proportional to the amount still present in the fibres.

If the amount of activity within the fibres is y and the amount leaving in the infinitesimal time interval dt is dy then we have

$$\frac{dy}{dt} = k \cdot y \text{ or } y/y_0 = e^{-\frac{t}{k}} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

(y_0 is the amount of activity present at zero time).

The amount of activity leaving the fibres between $t = 0$ and $t = t_1$ is $y_0 - ye^{-\frac{t_1}{k}}$

Between $t = t_1$ and $t = 2t_1$ the amount leaving is:

$$\left(y_0 - y_0 e^{-\frac{2t_1}{k}}\right) - \left(y_0 - y_0 e^{-\frac{t_1}{k}}\right) = y_0 e^{-\frac{t_1}{k}}\left(1 - e^{-\frac{t_1}{k}}\right).$$

Between $t = 2t_1$, and $t = 3t_1$ the amount leaving is $y_0 e^{-\frac{2t_1}{k}}\left(1 - e^{-\frac{t_1}{k}}\right)$. In equally long subsequent periods the amounts of activity lost from the fibres evidently form the series:

$$y_0\left(1 - e^{-\frac{t_1}{k}}\right) e^{-\frac{t_1}{k}},\ y_0\left(1 - e^{-\frac{t_1}{k}}\right) e^{-\frac{2t_1}{k}} \ldots\ldots\ y_0\left(1 - e^{-\frac{t_1}{k}}\right) e^{-\frac{nt_1}{k}}.$$

Thus we may form the function

$$z = y_0\left(1 - e^{-\frac{t_1}{k}}\right) e^{-xt_1} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

$$\text{or } \log z = \log\left[y_0\left(1 - e^{-\frac{t_1}{k}}\right)\right] - \frac{xt_1}{k} \cdot \frac{1}{2.3} \text{ from which } \frac{d \log z}{d[x \cdot t_1]} \cong \frac{1}{2.3k}. \quad (3)$$

In other words, if the logarithms of the amounts of activity washed out in subsequent, equal periods is plotted against time, the slope of the resulting straight line can be used to determine k which in its turn is a measure of the rate at which Na^{24} escapes from the muscle fibres.

In these experiments, the diffusion out of Na^{24} from the interspaces is superimposed on the escape of Na^{24} from the cells. The diffusion from the interspaces, however, is a much more rapid process and after the lapse of some time it will no longer contribute measurably to the amount of activity washed out. As a first approximation we may regard the muscle as a thin sheet of homogeneous tissue exposed on both sides to a solution in which the Na^{24} concentration is practically zero. In such a system (cf. JACOBS 1935) the total amount $Q_{0,t}$ escaping in the time interval $t = 0$ and $t = t$ is determined by

$$Q_{0,t} = U_0 AH\left[1 - \frac{8}{\pi^2}\left(e^{-\frac{\pi^2 Dt}{4H^2}} + \frac{1}{9} e^{-\frac{9\pi^2 Dt}{4H^2}} + \ldots\right)\right] \quad \ldots\ldots \quad (4)$$

Except for very short periods this expression reduces to:

$$Q_{0,t} = U_0 AH\left[1 - \frac{8}{\pi^2} \cdot e^{\frac{\pi^2 DA}{4H^2}}\right] \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (5)$$

where U_0 is the initial concentration of the substance in the system, A is the exposed area, and H is the distance from the midplane of the tissue to the exposed surfaces. D is the diffusion coefficient.

In analogy to the considerations concerning the escape of Na^{24} from the fibres we find that the amount diffusing out of the interspaces in equal, subsequent periods decreases according to an exponential function:

$$q = U_0 AH \frac{8}{\pi^2}\left(1 - e^{-\frac{\pi^2 Dt_1}{4H^2}}\right) e^{-\frac{\pi^2 Dxt_1}{4H^2}} \quad \ldots\ldots\ldots\ldots \quad (6)$$

where t_1 is the duration of the periods and x the number of periods.

This equation should hold even if the tissue from which diffusion occurs is not homogeneous but consists of a meshwork of interspaces between much less permeable fibres. H then denotes the mean path and A the mean area of diffusion from the midplane to the surface of the muscle.

Thus it is seen that the process of washing out active ions from the muscle can be described as the sum of two exponential functions.

The two functions are separated in the same way followed when determining the individual half lives of two radioactive substances from measurements of their mixture by plotting the logarithms of the activities against time. As mentioned above nearly all the Na^{24} originating from the interspaces will have disappeared after one hour. Therefore a straight line drawn through the points obtained after the lapse of one hour is a measure of the rate of escape of Na^{24} from the fibres. The share of this process at the respective times is then subtracted from each value obtained during the first hour of washing. As the washing periods are not equally long, it is necessary to apply a correction to those interspace activities which are found during 10 min. and 20 min. periods, so that all interspace activities correspond to 2 min. washing periods.

The correction can be calculated from formula (6) p. 237 which can be reduced to $q = C\ (1 - e^{-\lambda t_1})\ e^{-\lambda t_1 x}$. We have then:

$$q_{10'}/q_{2'} = \frac{1 - e^{-10\lambda}}{1 - e^{-2\lambda}} \cdot e^{4\lambda}$$

if the half renewal time of interspace Na in 2 min. λ is 0.347; q_{10}/q_2 is then 7.76. As we have already divided q_{10} by 5, the factor to be applied to the interspace activity from the 10 min. period is 0.645. Within the experimental error the resulting points fall on a straight line the slope of which can be used for calculating the rate of Na-diffusion through the interspaces.

Results.

1. The fact that the experimental data, when plotted on a semilogarithmic scale, can be represented by two straight lines may be taken as evidence for the correctness of the above assumptions concerning the nature of the washing out of activity from the muscle.

Strictly speaking the washing out experiments show only that the Na^{24} washed out originates from two different phases which are not equally accessible to Na-exchange. Nevertheless, there can be no doubt that a considerable part of the exchangeable Na must be located in the fibres. This appears from the following calculation. A freshly dissected sartorius muscle was placed for 2.5 hours in 2 ml of Ringer solution containing Na^{24} (5.8×10^5

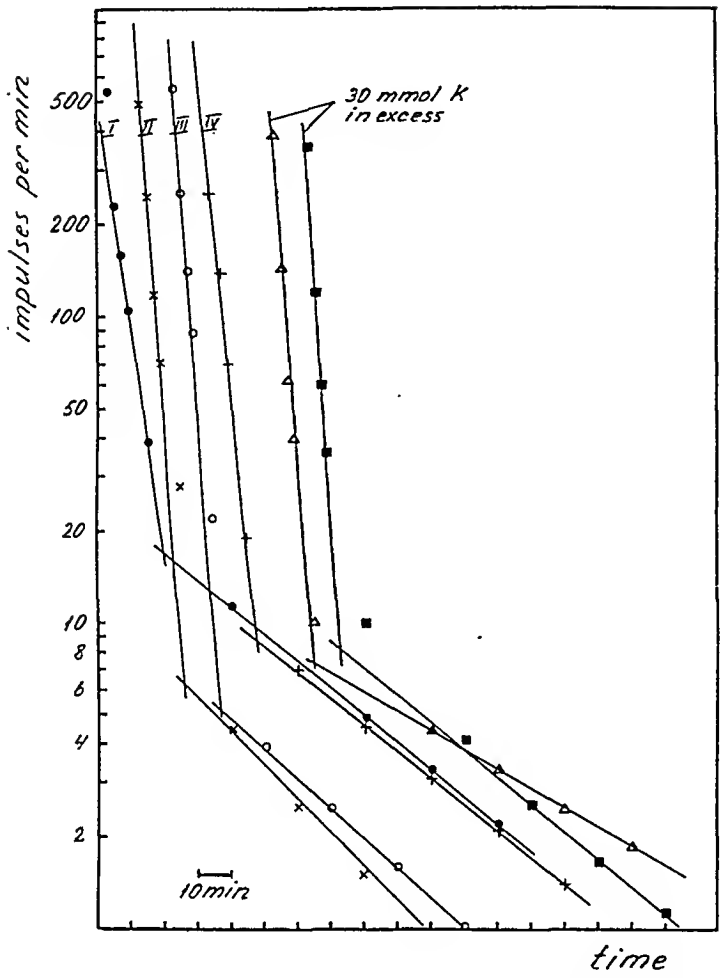

Fig. 3. The washing out of Na^{21} from the isolated frog sartorius (see text).

imp./min.). Then the muscle was taken up, dipped for one second in active Ringer, and the adhering water removed immediately with soft filter paper. Finally, the muscle was weighed on a torsion balance (weight 84.5 mg) and ashed in a platinum boat. The ash was dissolved in 1.60 ml of water; $\frac{1}{40}$ of this solution showed an activity of 3,180 imp./min., which means that the total muscle contained 127,300 imp./min. Estimating the interspaces at 13 % of the muscle weight, the interspaces must have contained $5.8 \times 10^6 \times \frac{13}{100} \cdot \frac{84 \cdot 5}{1000} = 63{,}700$ imp./min. $127{,}300 - 63{,}700 = 63{,}600$ imp./min. thus originate from the fibres. A similar

experiment on a muscle weighing 73 mg gave 65,000 imp./min. within the fibres. As will be shown later, however, from the Na-diffusion point of view a certain fraction of the fibre volume behaves like the interspaces proper.

2. *The rate of Na^+-escape from the fibres.* Fig. 3 shows the results of a series of washing out experiments where the muscles had previously been soaked in active (Na^{24}-)Ringer for two hours. All experiments were performed at room temperature. In two experiments the Ringer solution contained 30 mmol KCl/l in excess over the normal KCl-concentration. The abscissa represents time in minutes and the ordinate the activity in a logarithmic scale. The activity is given as imp./min. in 100 μl washing fluid.

The distribution of the washed out activity between the part originating from the interspaces and that coming from the fibres has been described above. It is seen that the points fit quite neatly on two straight lines. From the slope of the less steep line the time for half renewal of sodium in the fibres is found to be between 26 and 47 minutes. The mean value obtained on 18 different muscles is 34 min. (one aberrant value, 70 min., was discarded). The presence of excess K in the washing fluid seems to have little or no influence on the rate of Na-renewal in the fibres.

If the experiments are performed at 1° C instead of 20° the renewal rate is considerably lower; four muscles gave 70 minutes as the mean half renewal rate, with a deviation of only $\pm$ 10 % from this value.

3. *The rate of Cl^--escape from the fibres.* The washing out of Cl^{38} from the muscles cannot be described as a diffusion from one phase either, however, the experimental figures fit fairly well on two straight lines. The less steep line is regarded as a measure of the renewal rate of the Cl-ions in the muscle fibres.

Cl^- was found to leave the fibres considerably faster than Na^+. Therefore, after the first 5 washings which lasted two minutes each the washing periods were 10 minutes. Fig. 4 shows the rate of washing out of Cl^- from the fibres. (The steeper line indicating the washing out of the interspaces is omitted in this figure.) The time of half renewal is about 10 min. Although the time of half renewal of Na in the interspaces (about 2 min.) and the corresponding value for the fibres (34 min.) are sufficiently different to give a clear resolution in two straight lines, the problem is more difficult in the case of Cl^-. In the interspaces Cl^- is renewed at about the same rate as is Na^+, whereas the fibre Cl^- is renewed

at about a five times slower rate. Nevertheless, it is obvious that in the fibres Cl is renewed much faster than is Na^+.

4. *The estimation of the interspace volume.* In many of the experiments the two sartorii from one frog were equilibrated with

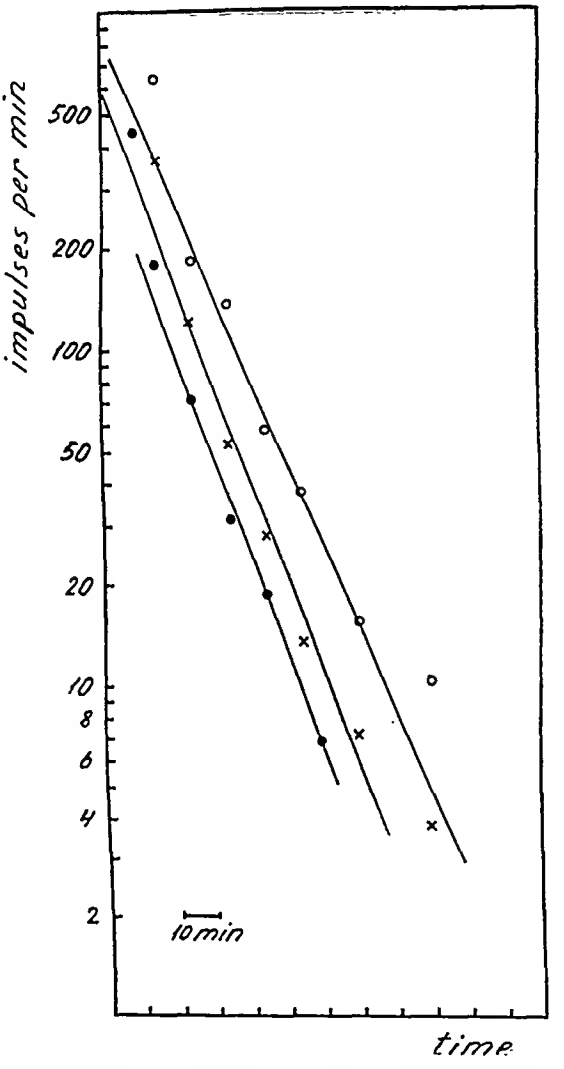

Fig. 4. The washing out of Cl^{38} from the isolated frog sartorius (see text).

active Ringer. One of them was then washed out whereas the other one was wiped dry with filter paper and ashed. The Na^{24} content of the ash may be regarded as a fair measure of the Na^{24} present in the washed muscle before washing. (Apparently this value could be found more directly by summing up the activity washed out, but the muscle to be washed was not entirely

freed from adhering active Ringer because the treatment with filter paper might damage the fibres.)

By extrapolation backward it is possible to calculate the total amount of activity washed out from *the fibres*. This value is subtracted from the total activity found in the parallel muscle. (Parallel muscles as a rule have the same weight within 1—2 mg.) The resulting activity must originate from the interspaces. Knowing the activity of the soaking solution and assuming the interspace fluid to be identical with Ringer we can calculate the interspace volume.

Table 1.

No. of experiment	Weight of muscle	Total activity in "living fibres" at start of washing (calculated)	Total activity in parallel muscle corrected for difference in weight	Activity per ml soaking solution	Total activity in interspaces	Apparent interspace volume % of muscle weight
I, 12/3	78	5 730	39 000	1.58×10^6	33 270	27.0
III, 12/3	53	5 600	44 100	2.1×10^6	38 500	34.6
1, 12/3	67	6 060	36 100	1.58×10^6	30 040	28.3
3, 12/3	48.5	4 870	35 000	2.1×10^6	30 130	29.6
1, 13/3	56	4 850	18 650	0.75×10^6	13 800	32.2
3, 13/3	58	3 080	12 400	0.5×10^6	9 320	32.1

These values are all much higher than the generally accepted 13 %. Thus there seems to be a part of the muscle anatomically belonging to the fibres, which — under the present experimental conditions — exchanges its Na^+ just as readily as do the interspaces proper.

This may mean that in the rather prolonged experiments some of the fibres loose their specific, low permeability to Na^+ during soaking. Such fibres will then exchange most of their K with Na and from then on they will behave as part of the interspaces. A more reasonable explanation is, however, that the surface layer of the fibres, the sarcolemma, is situated outside the cell membrane. It is also possible that a certain amount of Na ions is bound at the fibre surface either as complex or as counter ions for some high-molecular acid substance like hyaluronic acid. The presence of bound Na in the interspaces is indicated in the experiments of MOND and NETTER (1932). In view of the possibility that there may be a higher Na-concentration in the interspaces than in

Table 2.

Concentration in fibre water.

	Na mmol	K mmol
III, 12/3	12	125
I, 12/3	11	114
I, 12/3	6.5	125

Ringer solution, the calculated apparent interspace volumes should be regarded as rough estimates, only.

5. *Calculation of the Na- and K-concentration in the fibre water.* Knowing the apparent interspace volume (including dead cells) we can calculate the amount of fibre water in the still living fibres.

Taking muscle 1, 13/3 (see table 1) as an example we find the apparent interspaces to be 35 % of the muscle; if the rest of the muscle is taken to have 23 % dry substance the fibre water volume is 28 μl.

The activity inside the living fibres was found to be 4,072 imp./min. or 146,000/ml. The activity of the soaking solution was 7.5×10^5 imp./min., ml. This means that a 23 mmolar Na solution inside the fibres would have been in equilibrium with the outside solution as to specific activity. The time of half renewal of the Na in the fibres being in this case 37 min., two hours soaking should have brought the inside activity rather close to the equilibrium so that the true Na-concentration in the fibres might have been only about 10 % higher than the calculated value.

The K-content of this muscle was 2.1 μmoles. Correcting for the interspace K, 2.04 μmoles must have come from the fibres. The K-concentration in the fibre water was thus 73 mmol/l. Similar calculations for muscle 3, 13/3 give 23 mmol/l Na and 71 mmol/l K in the fibre water of the "living" fibres.

These two muscles were soaked and washed with ordinary Ringer. Muscles which from the moment of excision were kept in "Ringer" with 30 mmol excess KCl give a considerably lower value for the intracellular Na (see table 2).

It must be admitted, however, that this way of calculating the intracellular Na-concentration is apt to yield too low figures. The line, the slope of which determines the renewal rate of Na in the fibres, is drawn through the points obtained from the last washings. If therefore the rate at which Na leaves the fibres decreases with time, the slope of the line will correspond to the

lower renewal rate, and the values for the activity present in the fibres at the beginning of the washing will turn out too low.

Discussion.

It is a simple matter to calculate the amount of Na leaving the fibres per unit time from the Na-half renewal time and the Na-content of the fibre water. If for instance the Na-concentration is 25 mmol/l and the half renewal time 30 min. this means that $\frac{0.693}{0.5} \cdot \frac{25}{1000} = 0.0346$ moles Na leave the fibres per hour. The first problem to be discussed is: How much of this sodium can be assumed to leave the fibres by simple diffusion as free ions?

We know that the Na-concentration in the fibre water is lower than in the Ringer solution; moreover there is an electric potential difference between the fibres and the surrounding fluid, the interior of the fibres being negative relative to the bathing fluid. Both factors tend to increase the influx of Na-ions and to reduce the outflux. The relation between the forces acting on the ions and the resulting diffusion rates can be gained as follows:

The force acting on one single Na^+ ion is

$$-\frac{1}{N_0} \cdot \frac{d\bar{\mu}}{dx} = wG \quad \ldots\ldots\ldots \quad (1)$$

where N_0 is Avogadro's number, $\bar{\mu}$ is the electrochemical potential of the sodium ion, w is the mean rate of movement and G is the friction acting on one ion under the influence of unit force. X_0 is the thickness of the membrane. The amount $\frac{dn}{dl}$ of Na which crosses an area A perpendicular to the direction of diffusion per unit time is

$$\frac{dn}{dl} = w \cdot C_{Na} \cdot A \quad \ldots\ldots\ldots \quad (2)$$

where C_{Na} is the Na concentration at A (we consider a steady state where $\frac{dn}{dl}$ is constant and C_{Na} has a definite value for all values of X). Combining (1) and (2), we obtain

$$M = \frac{dn}{dl} = -\frac{A}{G \cdot N_0} \cdot C_{Na} \frac{d\bar{\mu}}{dx} = -\frac{ART}{G \cdot N_0} \left(\frac{dC_{Na}}{dx} + C_{Na} \frac{F}{RT} \cdot \frac{D\psi}{dx} \right)$$

(ψ is the potential, T the absolute temperature, R the gas constant and F Faraday's number).

We may now introduce Poisson's equation:

$$\frac{\delta^2(\varepsilon\psi)}{\delta x^2} + \frac{\delta^2(\varepsilon\psi)}{\delta y^2} + \frac{\delta^2(\varepsilon\psi)}{\delta z^2} = -4\pi\varphi$$

where x, y and z are the space coordinates, ε is the mean dielectricity constant of the membrane and φ the net charge (charge of cations — charge of anions). As the potential is not assumed to change in the y and z directions, the expression reduces to

$$\frac{d^2(\varepsilon\psi)}{dx^2} = -4\pi\varphi.$$

At no point in the membrane can there be any difference between the number of positive and negative charges and thus we find

$$\frac{d^2(\varepsilon\psi)}{dx^2} = 0 \text{ or } \frac{d\psi}{dx} = \text{constant} \left(= \frac{\psi_0}{x_0}\right) \quad \ldots\ldots \quad (4)$$

where ψ_0 is the potential difference across the membrane.

(3) and (4) give

$$M = -\frac{ART}{GN_0}\left(\frac{dC_{Na}}{dx} + C_{Na} \cdot \frac{F}{RT} \cdot \frac{\psi_0}{x_0}\right)$$

from which

$$C_{Na} = -\frac{MGN_0X_0}{AF\psi_0} + J \cdot e^{-\frac{F}{RT}\psi_0 \cdot \frac{x}{x_0}} \quad \ldots\ldots\ldots \quad (5)$$

J is an integration constant.

To eliminate J we have to define the boundary conditions for the diffusion. If we wish to know the total flux of Na-ions which passes from the inside to the outside of the membrane we must put C_{Na} equal to $C_{Na(i)}$ for $x = 0$ and $C_{Na} = o$ for $x = x_0$. $C_{Na(i)}$ is the constant Na concentration in the fibres. (C_{Na} then denotes, not the Na concentration at a certain point within the membrane, but the concentration of those Na-ions the destination of which is the outside solution!)

Elimination of J and rearrangement lead to the following expression:

$$M = \frac{AF\psi_0}{GN_0X_0} \cdot \frac{C_{Na(i)}\, e^{-\frac{F}{RT}\psi_0}}{1 - e^{-\frac{F}{RT}\psi_0}}.$$

The diffusion coefficient D is defined as $\frac{RT}{GN_0}$; inserting this ex-

pression we get

$$M = \frac{AF\psi_0 D}{RTX_0} \cdot \frac{C_{Na(i)} \cdot e^{-\frac{F}{RT} \cdot \psi_0}}{1 - e^{-\frac{F}{RT} \cdot \psi_0}} \quad \ldots\ldots\ldots\ldots (6)$$

If a Donnan distribution of the K-ions is established across the membrane (BOYLE and CONWAY l. c.) we may substitute $\frac{RT}{F} \ln \frac{a_{K(i)}}{a_{K(y)}}$ for ψ_0, where $a_{K(i)}$ and $a_{K(y)}$ denote the thermodynamic activities of K in the inside or the outside solutions, respectively.

$$M_{out} = \frac{AD \ln \frac{a_{K(i)}}{a_{K(y)}}}{X_0} \cdot \frac{C_{Na(i)} \cdot a_{K(y)}}{a_{K(i)} - a_{K(y)}} \quad \ldots\ldots\ldots\ldots (7)$$

The ratio between influx and outflux of Na-ions across the membrane is determined by

$$\frac{M_{out}}{M_{in}} = \frac{\ln \frac{a_{K(i)}}{a_{K(y)}} \cdot \frac{C_{Na(i)} \cdot a_{K(y)}}{a_{K(i)} - a_{K(y)}}}{\ln \frac{a_{K(y)}}{a_{K(i)}} \cdot \frac{C_{Na(y)} \cdot a_{K(i)}}{a_{K(y)} - a_{K(i)}}} = \frac{C_{Na(i)} \cdot a_{K(y)}}{C_{Ka(y)} \cdot a_{K(i)}} \quad \ldots\ldots\ldots (8)$$

On the assumption that the ionic strengths of the fibre water and the intercellular water are the same, the expression reduces to

$$\frac{M_{out}}{M_{in}} = \frac{C_{Na(i)} \cdot C_{K(y)}}{C_{Na(y)} \cdot C_{K(i)}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (9)[1]$$

This indicates clearly that the amount of Na which can diffuse out as free ions from the fibre water must be very small compared with the amount which diffuses in.

If for instance $C_{Na(i)} = 20$ mmol/l $C_{Na(y)} = 115$ mmol/l $C_{K(i)} = 125$ mmol and $C_{K(y)} = 32.5$ mmol/l, we have $\frac{M_{out}}{M_{in}} = \frac{20 \times 32.5}{116 \times 125} = 0.045$. In spite of this, the Na-concentration of the fibre water increases but slowly. Thus it is quite impossible that the renewal rate for Na in the fibres, as determined with the tracer method, can be a measure of the diffusion out of free Na-ions.

Is, then, the renewal of Na in the fibres a measure of the rate

[1] In a paper just received (CONWAY 1947) an identical expression for the relation between the amounts of an ion diffusing in and out of the muscle is derived. The treatment given here has, however, the advantage that both the diffusion in and the diffusion out can be described by a common diffusion coefficient D, whereas CONWAY operates with one constant for diffusion in and another for diffusion out.

at which Na-ions are actively extruded from the fibres? Most authors seem to agree that a Na-extrusion does take place although there is still dispute as to the extent of this process.

In discussing this question we may calculate the work necessary to perform an extrusion of Na from the fibres at the rate at which Na actually crosses the fibre membrane. In this way we can at least find out whether it is energetically possible that Na leaves the fibres exclusively as a result of active transport. If the Na-concentration in the fibres and the potential across the membrane are constant it would mean — according to the active transport concept — that one mole Na is transported out for every mole that diffuses in. Under such conditions the work performed per hour and per kg fibre water is $M_{Na(out)} = (\bar{\mu}N_{a(y)} - \bar{\mu}_{Na(i)})$ where $M_{Na(out)}$ is the amount of Na (in moles) which leaves 1 kg fibre water per hour and $\bar{\mu}_{Na(y)}$ and $\bar{\mu}_{Na(i)}$ are the electrochemical potentials of Na in the two media. $\bar{\mu}_{Na(y)} - \bar{\mu}_{Na(i)}$ is equal to $RT\ (\ln C_{Na(y)} - \ln C_{Na(i)} + \ln C_{K(i)} - \ln C_{K(y)})$ on the assumption that the ionic strength inside and outside is the same.

We may take experiment III 12/3 as an example (see table 2): half renewal time 34 min. $C_{Na(y)}$ 115 mmol. $C_{Na(i)}$ 12 mmol. $C_{K(i)}$ 125 mmol. $C_{K(y)}$ 32.5 mmol.

The energy required would be

$$0.012 \times 1.225 \times 1.985 \times 293 \times 2.3 \times 2.567 = 51 \text{ cal/hour.}$$

In the experiments with normal Ringer as washing fluid the potential across the membrane has probably been lower than calculated from the K-concentrations. According to BOYLE and CONWAY (1941) the potential is as a rule about 60 mV, corresponding to the proportion $\frac{C_{K(i)}}{C_{K(y)}} \cong 10$. Using this proportion instead of that actually found, the energy required for the Na-extrusion in experiment 1, 13/3 amounts to 59 cal/hour, kg. According to CONWAY (1946) the metabolism of resting muscle from winter frogs is about 175 cal/hour. This means that more than 30 % of the energy output of the muscle would be consumed by the active Na-extrusion even within 100 % efficiency of the energy transfer from metabolic processes to active transport.

Moreover, for several reasons, the methods used here for the estimation of the Na-content of the fibres and the half renewal time of Na tend to give too low results. The true amount of Na leaving the fibres is therefore probably higher than assumed in

the calculations, and the computed energy is therefore a minimum value.

These considerations show that, although the renewal of Na in the fibres as measured with the tracer method *may* be a measure of the active extrusion, there are indications that the method gives too high values for the transport work. As pointed out by one of us (USSING, 1948), an exchange of sodium ions across the membrane (one ion going out for each ion going in) may, at least theoretically, take place without consumption of metabolic energy. A necessary condition for such an exchange is that Na travels not as free ions but as uncharged complex molecules.

Such a hypothetical exchange system can be visualized simply as a Na-impermeable monolayer containing scattered anions of a substance which forms a stable complex with Na. Due to thermal movements, these complex molecules will sometimes come into contact with the outside medium and sometimes with the inside medium. If the inside solution contains Na^{24} ions these may exchange with Na^{23} in some of the complex molecules and when these molecules later touch the outside solution, Na^{24} will leave the complex in exchange for Na^{23}.

In its ideal form such a mechanism will always take up one Na ion when it gives off another, so that no net change in the Na-concentrations on either side of the membrane need take place. It is necessary for such an "exchange diffusion" that the complex former has a much higher affinity for Na than for K, because else the inside K would exchange with outside Na. It must be remembered, however, that also the active extrusion of Na from the cells requires the formation of a specific Na-complex (compare USSING, 1948). The mechanism of active salt uptake as found in the frog skin is known to prefer Na to K, in fact no K uptake has so far been observed (KROGH 1937).

To summarize it can be said that the steady outflux of Na-ions from the living muscle fibres is only to a negligible extent due to diffusion of free ions.

Active extrusion is bound to account for part of the outflux; moreover, it is likely that part of the apparent active extrusion is due to exchange diffusion.

The authors are greatly indebted to the Rockefeller Foundation for grants which have made this investigation possible.

Summary.

1) A method is described for the determination of the rate of exchange of ions across the fibre membrane of isolated frog sartorii.

2) The Na^+ of the fibres is renewed at a fairly high rate, the time of half renewal being about 30 minutes. The rate of exchange is not much influenced when the K-concentration of the bathing fluid is increased from that of Ringer to 30 μ eqv./l.

3) The exchange of Cl^- across the fibre membrane seems to proceed at a still higher rate than does the exchange of Na^+.

4) An expression is derived which describes the flux of free ions across a membrane when the ions are acted upon simultaneously by a concentration gradient and an electric potential gradient.

5) It is concluded that the outflux of Na ions from the muscle fibres cannot be due to diffusion of free ions. Although it cannot be excluded that the outflux of Na^+ from the fibres is solely due to active transport, there are strong indications that at least a fraction of the Na^+ leaves the fibres by an exchange diffusion process.

6) Through an example it is demonstrated how an exchange diffusion system may be brought about.

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A Vitamin E Free Diet for Guinea Pigs.

By

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The exact study of an avitaminosis requires a basal diet lacking the vitamin to be investigated, but adequate in all other respects. As vitamin E is so widely distributed in plant tissue, it is very difficult to compose a vitamin E free diet for a herbivorous animal like the guinea pig. Therefore one has tried to destroy the vitamin E by adding an excess of an oxidation agent, generally ferric chloride, to the diet in question (GOETSCH and PAPPENHEIMER 1931). This procedure, however, has many disadvantages. The oxidation agent cannot be removed, and furthermore one cannot be sure that it has penetrated all the cell membranes, thus destroying the vitamin quantitatively. Finally one does not know the part played by the oxidation agent in the production of the changes observed in the animals. Another possibility of eliciting vitamin E deficiency is to inactivate the vitamin E in the intestine by feeding large amounts of cod liver oil or rancid fat (AGDUHR 1926, MACKENZIE et al. 1941, MATTILL and COLUMBIC 1942).

None of these methods permit quantitative experiments, as these would require a to the greatest possible extent synthetic diet. However, it has proved very difficult to compose a diet of this kind, as an adequate diet for guinea pigs, besides all known vitamins, has to contain certain other as yet unknown factors. In 1939 ELVEHJEM and coworkers, and CANNON and EMERSON showed that the so called grass juice factor was indispensable for

the normal growth of guinea pigs. This factor is a water soluble, relatively heat stable substance, which is most easily obtained from grass and lettuce, but which is also present in yeast. Soon thereafter it was shown that a diet consisting chiefly of casein and dextrin to which the grass juice factor was added, was inadequate for guinea pigs (KOHLER, RANDLE et al. 1939). All the animals reared on this diet developed acute gastric ulcers after 3—10 weeks, leading to death in 2—3 days. When the diet was supplemented with oats, the animals developed no ulcers. However, this ulcer factor was not analysed further. It has also been shown that there are still other indispensable food factors in an adequate guinea pig diet (KUIKEN et al. 1944, SOBER et al. 1942, WOOLLEY 1942).

Material and Methods.

Owing to the war the recent literature was not available, and the above mentioned investigations on the nutrion of guinea pigs could unfortunately not be considerated at the beginning of this experiments in 1944. Therefore, when trying to compose a vitamin E free diet for guinea pigs I started with BACHARACH's vitamin E free diet for rats (BACHARACH 1938). The animals, however, refused to eat this food. After the sugar content had been diminished and starch added, they ate it. The variations listed in Table I were then tried. In all more than 200 guinea pigs had to be used for the elaboration of a suitable diet.

Table I.

Diet	1	2	3	4	5	6	7	8	9	10	11	12
Wheat starch ...	200	600	500	500	450	275	550	590	600	370	350	340
Sucrose (commercial)	120	0	0	0	0	0	0	0	0	0	0	0
Cellulose	140	0	100	100	100	275	0	50	50	50	50	50
Casein	200	200	200	180	180	180	180	180	180	180	150	150
Yeast	80	80	80	80	80	80	80	80	80	80	100	120
Lard	220	80	80	100	150	150	150	60	50	80	70	60
Extr. of alfalfa...	0	0	0	0	0	0	0	0	0	200	200	200
Salt mixture	40	40	40	40	40	40	40	40	40	40	40	40
Agar-agar	0	0	0	0	0	0	0	0	0	0	40	40
Dry substance...	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000
Water	3 000	3 000	3 000	4 000	3 000	2 000	3 000	3 000	3 000	3 000	3 000	3 000

Vitamin A: 28 000 I. U. per kg. of dry substance ("Jecototal" Astra)
Vitamin D 6 000 I. U. per kg. of dry substance ("Jecototal" Astra)
Vitamin K, Sodium-2-methyl-1,4-naphtohydroquinone-diphosph. 0.02 gms. per kg. of dry substance
Vitamin C, Ascorbic acid 25 mgs. per animal and day.

The constituents of the diets have been prepared as follows:

Wheat starch and casein: The commercial products have been extracted with 95 % benzolated alcohol, 5 extractions by heating to the boiling point, using twice the weight of alcohol each time.

Cellulose: Refined wood powder (commercial).

Yeast: Fresh, pressed brewer's yeast was ground through a grating and dried in an exsiccator at 30° C, and then extracted 5 times with ether, each time with twice its weight of ether.

Lard: Factory made, refined lard, which contains minimal amounts of vitamin E (CHIPAULT et al. 1945).

Extract of alfalfa: 25 kgs. of alfalfa powder were extracted with 400 litres of water for one hour at 90° C, the mixture beeing stirred the whole time. After filtering in a filter press, it was concentrated to a thick extract by evaporation, and dried in vacuum. Then the dried extract was extracted with ether 5 times, each time with twice its weight of ether.

Salt mixture: The salt mixture of OSBORNE and MENDEL 1918 was prepared according to the instructions in the United States Pharmacopœia.

Agar-Agar: Agar-agar was pulverized and sifted to powder no. 30, then extracted with ether 5 times, each time with twice its weight of ether.

The diet was prepared as follows. The dry substance except the yeast and the lard was mixed together, and then mixed with the just boiled water. The starch was thereby hydrolysed, and the mixture assumed the consistency of a mucilaginous porridge. When the porridge had cooled off, the melted lard, the yeast, and the vitamins A, D, and K were kneaded carefully into it. The prepared food was kept in an ice-chest, however, not longer than 4 days.

So as to obtain some wearing of the teeth, each animal was given up to a maximum of 2 gms. of filter paper daily. The animals were kept 5 in each cage, the cages measuring 50 × 60 × 40 cms., and provided with a grated floor made of galvanized wire, with $^1/_2$" meshes, so as to prevent coprophagia. The animals were fed once daily at a set time, and were weighed every 7th day before the feeding. The ascorbic acid was administered by letting the animals drink an ascorbic acid solution from narrow necked bottles, suspended upside down, and easily accessible in the cage. Two such bottles were placed in each cage. Daily prepared 0.25 % ascorbic acid solution was given, calculating with a consumption of 10 mls. per animal and day. Apart from this the animals got nothing to drink.

Results.

None of the diets 1—7 (Table I) could be used, as the animals ate little and developed digestive disturbances, chiefly strong meteorism, and died within 2—3 weeks. Diet 8 was better, they ate more but still developed considerable meteorism. They liked diet 9 but

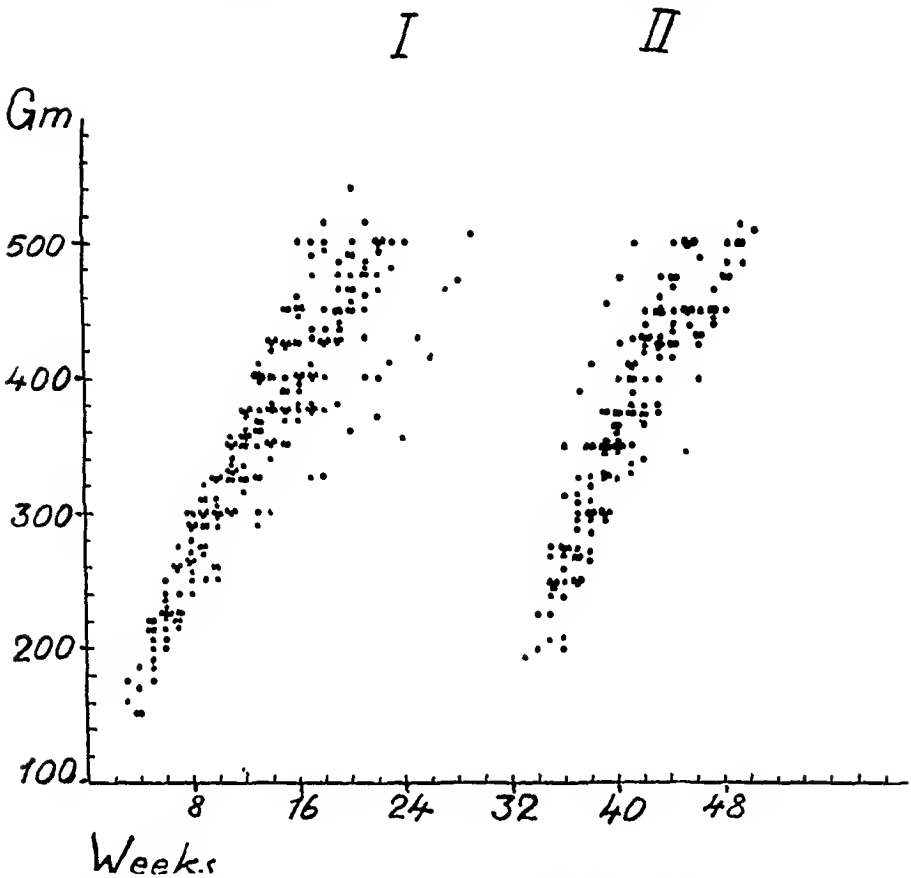

Fig. 1. A compilation of the weight curves from: I. 15 guinea pigs reared on a natural diet of hay and turnips. II. 14 guinea pigs reared on diet 12 + 1.2 mgs. tocopherol each per day administered by tube as a solution of disodium dl-α-tocopherylphosphate (kindly supplied by Hoffman-La Roche Company).

in this case growth was impaired. After adding the grass-juice factor (diet 10) by supplementing the food with extract of alfalfa powder, the growth was satisfactory. After 3—4 weeks, however, most of the animals began to lose weight, and then they died within 2—3 days from haemorrhages from multiple up to confetti sized, superficial ulcerations in the gastric mucosa. In exceptional cases such ulcerations were also found down in the intestines. In a few cases solitary ulcers of chronic character developed.

As was mentioned above KOHLER et al. 1939 had supposed that seeds contained an anti-ulcer factor, because the animals the diet of which was supplemented with oats developed no ulcers. After several failures in getting a vitamin E free extract containing the anti-ulcer factor, from grass and other plants, I found that agar-agar could also prevent the development of gastric

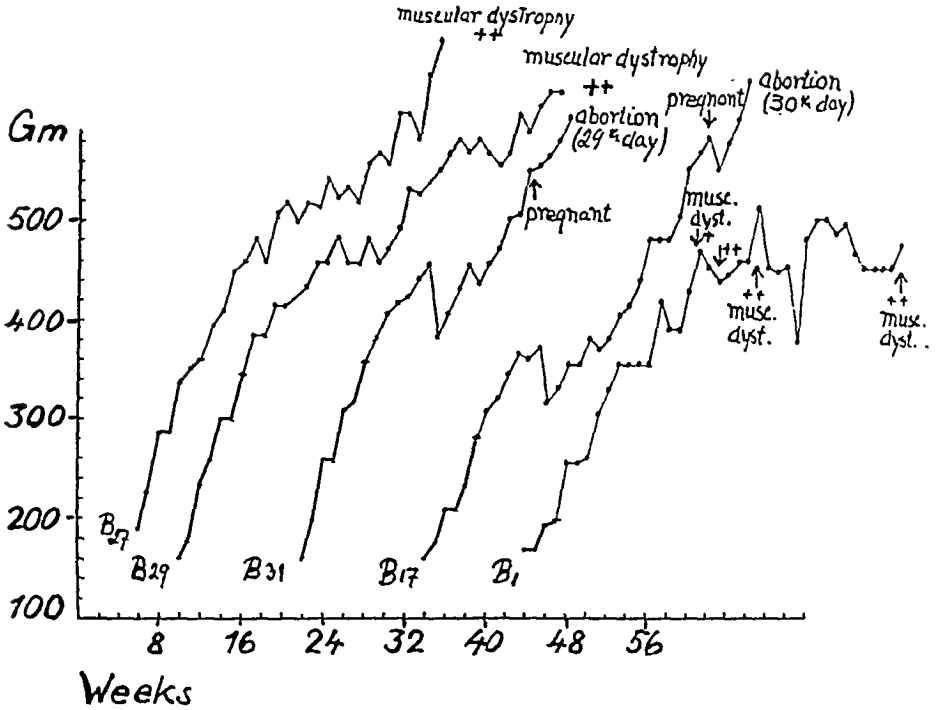

Fig. 2. Weight curves from 5 animals, receiving diet 12 without the addition of vitamin E. The thick-drawn part of the curves represents the period when the animals were given hay and turnips, and the thin-drawn part the period when they were given diet 12 only.

ulcers. An additon of 4 % ether extracted agar-agar to the dry substance of the diet was necessary in order to prevent ulcers (diet 11). As the animals sometimes showed a tendency of shedding hairs, the addition of extracted yeast was increased to 12 % in diet 12.

Diet 12 has proved to be very suitable for vitamin E experiments on guinea pigs. When administering vitamin E separately and in sufficient amounts, the animals can be kept on this diet for years, showing normal reproduction and the same weight increase as animals kept on a natural diet of hay, turnips etc. (Fig. 1).

Without the administration of vitamin E, muscular dystrophy develops within $2^1/_2$—3 months. If the animals become pregnant after this time, abortion caused by alterations in the placenta occurs on the 20th—30th day (Fig. 2).

Summary.

A vitamin E low diet of the casein-dextrin type has been worked out. The vitamin B complex is administered as ether extracted

brewer's yeast, the vitamins A and D as a concentrated preparation from cod liver oil ("Jecototal", Astra), the vitamins C and K in the pure form, as ascorbic acid and sodium 2-methyl-1,4-naphtohydroquinone-diphosphate respectively. The grass juice factor was added by supplementing the diet with an ether extracted aqueous extract of alfalfa powder, and the development of gastric ulcers was prevented by adding ether extracted agar-agar. Essential unsaturated fatty acids were administered by giving refined lard which was prepared in factory scale from a large raw material, wherefore its vitamin E content was minimal.

I would like to express my appreciation to the Chief of Astra's central laboratory Mr. B. Sjögren, M. D. and Mr. Henry Larsson, thanks to whom the different constituents of the diets were prepared by A.B. Astra, Södertälje.

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Combined Action of Prolonged Exposure to Small Doses of Carbon Monoxide and Trichlorethylene.

By

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Some years ago we went into the question of the combined effect of carbon monoxide and trichlorethylene as a possible cause of an airplane accident. It was evident that a study of these actions would be of theoretical interest as both agents may cause a reversible narcosis of the central nervous system with unconsciousness as one symptom. The cause is most probably an inhibition of the metabolism in the nervous tissue, but this action is achieved in entirely different ways by the two agents.

Carbon monoxide reduces the supply of oxygen to the brain by being combined to the haemoglobin, and so inhibiting the latter's uptake of oxygen. Although other effects, such as inhibition of the oxyhaemoglobin dissociation, dilatation of the vessels etc. may be added to the first mechanism, the final effect is a reduced supply of oxygen to the tissues [DRINKER (1938), SJÖSTRAND (1942), von OETTINGEN (1944), FORSSMAN (1946), GULLBERG, SWENSSON and WOHLFART (1947)].

The effect of *trichlorethylene* on the central nervous system is the same as that of other aliphatic inhalation anesthetics, exerting its action directly on the cells of the central nervous system. Whether the mechanism is to be explained by an effect on cell permeability, by interfering with lipid solubility or surface activity, or by inhibition of cellular oxidation, the final result is probably a reduction of the metabolism of the nervous

tissues (QUASTEL 1943). Thus the sites or ways of action differ entirely for carbon monoxide and trichlorethylene. This implies a study of the possible combined action of these agents to allow of acquiring certain knowledge on the mechanism of synergism and of potentiation.

The problem set for this work was to study the combined action of carbon monoxide and trichlorethylene on mice, to try to increase the sensitivity of the methods used by keeping the concentrations of the two agents involved as low as possible, thus testing threshold effects, and finally to investigate the possibility of utilizing the methods adopted for studying the effect of low concentrations of carbon monoxide.

Experimental.

Method.

Material: White mice with a body weight varying from 18 to 22 g were used throughout, 277 animals in all, divided into 11 series.

Exposure to CO: About five mice belonging to the same experiment series were placed simultaneously in a widenecked bottle, holding a volume of c. 3 litres, and were exposed to carbon monoxide of various concentrations, c. 0.2 and 0.04 % by volume in air, for various lengths of time: 1—3 minutes, and 2—6 hours resp. The carbon dioxide formed, when the animals were shut up in the bottle, was adsorbed by soda lime placed on the bottom of the bottle under a metal netting. Determinations of the conc. of COHb were made spectrophotometrically.

Exposure to trichlorethylene: After a certain period of time the mice were transferred one by one into another widenecked bottle (2.5 litres), closed by a cork, passed by a glass staff bent into a hook at its lower end. In this hook a piece of gauze tissue was fastened. Trichlorethylene in a quantity of 27.9 mg/lit. air was pipetted on the tissue through a hole in the cork, which was closed completely by a rubber plug.

All experiments were performed at room temperature.

Anesthetic action: The anesthetic effect was tested by the Knoefel-Murrell method (for literature *vide* LINDGREN 1946), as modified by AHLMARK (to be published):

The bottle was rolled to and fro one meter in each direction, with a constant speed of 1 meter in 1.5 sec. The anesthetic action was divided into five phases or stages:

A) The animal slips for the first time.

B) Temporary supine position.

C) Supine position for 30 seconds (the bottle not rolled for one minute).

D) The animal rolls three times.

E) The animal rolls altogether passively, but can take a few steps when in a prone position ("parcel" stage).

The times for "induction" *i. e.* the latency times to the beginning of the different stages, were used as measures of the effect. Normally only a few animals will slip after a rather long time.

Doses: In order to increase the precision of the tests, *low* concentrations of both carbon monoxide: c. 0.2 and 0.04 % by volume in air, and trichlorethylene, 27.9 mg/lit, were used, where one agent alone was thought to show only a very slight action or no action at all.

For statistical analysis, it is of advantage from different points of view to have methods of testing significance etc. of normally distributed variates. In order to analyse the distribution of the induction times, 103 determinations on 103 animals are shown in figure 1, in 1 A the absolute values in min are given, and in 1 B the log values.

The times in absolute values are skewly distributed (1 A), and the log times are approximately normally distributed (1 B), which is in agreement of what is generally known of survival times (IPSEN and TOFT 1946), lethal times (*e. g.* BLISS and HANSEN 1939, GOLDBERG 1942), durations of anesthetic effect (GOLDBERG 1947) etc.

In consequence all statistical calculations were performed on log values; standard deviation, standard error of the mean, and significance of differences between means.

The percent and standard deviation (σ %) is computed from the standard deviation, calculated from log values (log σ), in the following way:

$$+\sigma\ \% = 100\ (\text{antilog}\ \sigma - 1) \qquad (1\ a)$$

$$-\sigma\ \% = 100\ [1 - \text{antilog}\ (-\sigma)] \qquad (1\ b)$$

An approximate way is suggested by COCHRAN (1938):

$$\sigma\ \% = 230.26 \times \log \sigma \qquad (2)$$

The formula (2) yields sufficiently exact answers up to standard deviations of 20—40 %, and is used throughout in this work.

The percent standard error of the mean ($\varepsilon_{\bar{x}}$ %) is calculated from the percent standard deviation as follows:

$$\varepsilon_{\bar{x}}\ \% = \frac{\sigma\ \%}{\sqrt{n}} \qquad (3)$$

and the standard error of the mean in absolute figures

$$\varepsilon_{\bar{x}} = \frac{\varepsilon_{\bar{x}}\ \% \cdot 100}{\bar{x}} \qquad (4)$$

where $\bar{x}$ = mean, n = number of cases.

Results.

Trichlorethylene.

Series a. The anesthetic action of trichlorethylene alone was investigated on 40 mice, the concentration of trichlorethylene in the bottle being 27.9 mg/litre air. The results are given in fig. 3.

It is seen from the graph that stages A and B form one group, the effect coming on in about 3.3—3.4 min., and stages C, D and E form another group, the effect coming after 6.9—7.6 min.

The tendency of increase in time between stage A and B was not significant, neither between C and D, whereas the other stages differed significantly from each other. This implies stages A (or B), C (or D) and E to be those principally to be observed.

These animals were used as *controls*, the values found being compared with the treated animals.

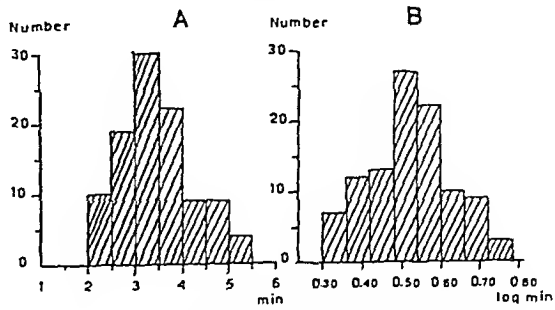

Fig. I. Distribution of Induction Times (n = 103).
A. Absolute Times in Minutes.
B. Log Times.

Combined Action of Carbon Monoxide and Trichlorethylene.

I. Short Exposure to Carbon Monoxide of Moderate Concentration:

Series b. Animals, 10 mice in each group, were exposed to carbon monoxide in a concentration of about 0.2 % CO by volume in air for periods of 1, 2 and 3 minutes, and were then immediately killed to determine spectrophotometrically the content of carboxyhaemoglobin, which was found to be c. 20 %.

Series c. In another series mice, 10 animals in each group, were thus exposed to carbon monoxide for 1, 2 and 3 minutes, and then tested for a possible anesthetic action of CO. No effect at all was found, the animals reacting quite normally when rolled.

Series d. In a third series mice, 10 animals in each group, were again exposed to carbon monoxide for 1, 2 and 3 minutes and were immediately transferred to another bottle, without time delay, and exposed to trichlorethylene: no statistically significant difference in time in the appearance of anesthetic effect was seen as compared to the effect obtained by trichlorethylene alone.

Thus exposure to carbon monoxide of 0.2 % by volume in air for 1—3 minutes, causing a concentration of COHb of c. 20 %, does *not* bring about any anesthetic action, as tested by rolling the animals. The anesthetic action of trichlorethylene is not affected by the animal being *acutely* exposed to carbon monoxide of 0.2 % by volume for a short period of exposure of 1—3 minutes.

II. Prolonged Exposure to Carbon Monoxide of Low Concentration.

Series e. In further experiments mice, 10—12 animals in each group, were exposed to carbon monoxide in a concentration of 0.04 % by volume in air for 2, 4 and 6 hours, the content of carboxyhaemoglobin being determined spectrophotometrically. The resulting concentration of COHb is shown in figure 2; its decomposition develops rapidly when the animals are placed in air and all CO is exhaled after 90 minutes.

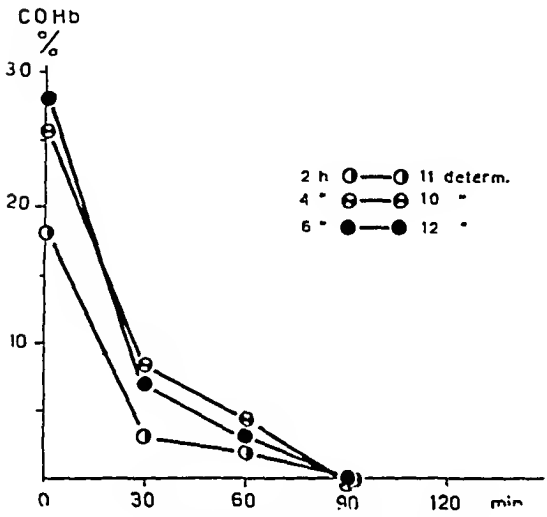

Fig. 2. Relation between Exposure to CO and Elimination.
3 groups of animals, exposed to c. 0.04 % CO in air for 2, 4 and 6 hours.

Series f. In another series animals thus exposed were tested for possible anesthetic action, no effect whatever was observed.

Series g. In a third series animals, 12—15 mice per group, were exposed to CO for 2, 4 and 6 hours, and immediately transferred to another bottle and exposed to trichlorethylene.

The results are given in figure 3.

In figure 3 A the absolute values are given, showing two facts:

1) The longer the exposure to CO, the higher the anesthetic action, *i. e.* the shorter the induction times.

2) The shorter the induction times, the slighter the absolute differences between the different stages, and the flatter the curve.

In figure 3 B the logarithmic values are given, and besides the fact that a longer exposure to CO causes shorter induction times the figure now shows another fact:

3) The curves are on the whole parallel to each other, thus the time of exposure to CO merely causing a shift of the whole curve.

A shift of a curve, based on logarithmic values, means a percentual change, being the same for all stages examined. The only exceptions are stages A and B, for 6 hours exposure, the times were so slight, however, 0.9 and 1.0 min. on an average, that the exceptions may simply be due to the impossibility of meas-

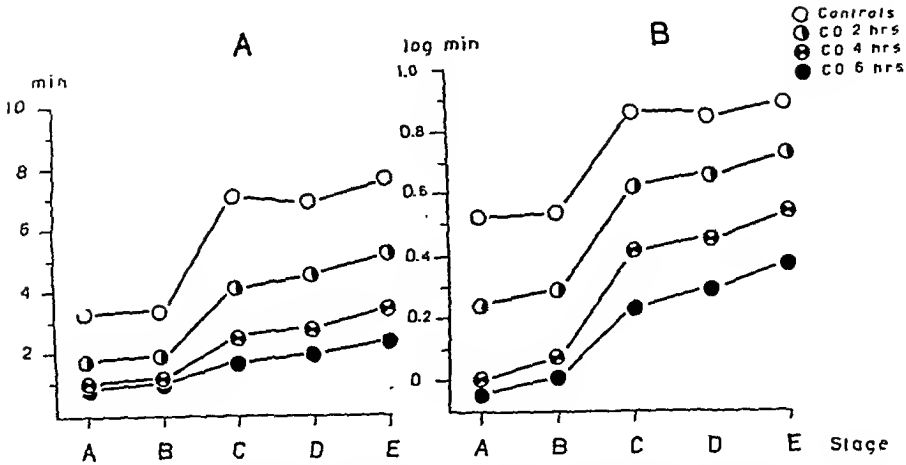

Fig. 3. Induction Times for Different Stages of Narcosis after Exposure to CO (0.04 % in Air) and to Trichlorethylene (Mean Values).

A. Induction Time in min.
B. Log time in min.
Stage A. Animal slips.
B. Temporary supine position.
C. Supine position for 30 secs.
D. Animal rolls three times.
E. Animal rolls passively ("parcel").

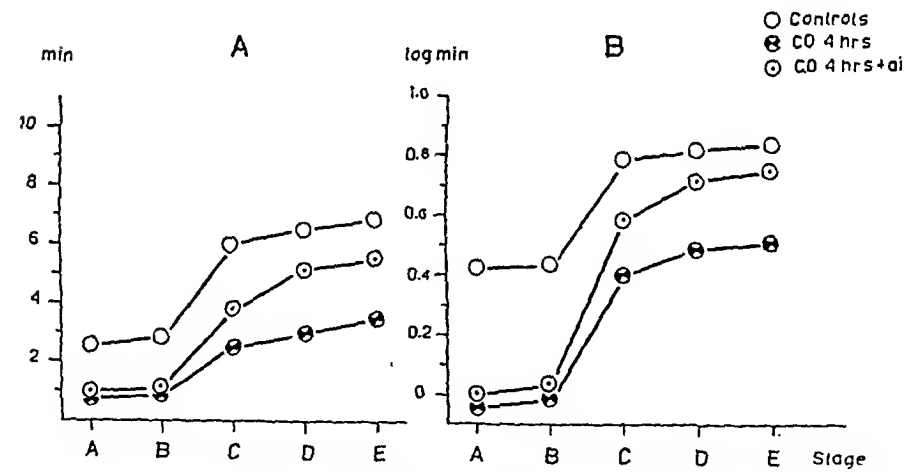

Fig. 4. Induction Times for Stages of Narcosis after Trichlorethylene (cp. fig. 3) (Mean Values).

uring exactly such short times under the conditions of the experiments.

An illustration is given in figure 5, where the induction times are plotted against time of exposure to CO, stages C, D and E are given. In A absolute values are given, and log values in B.

Exposure to carbon monoxide for a certain period of time thus causes a decrease in induction time, proportional to the time

of exposure. A logarithmic relationship was found, which denotes the induction time to be lowered by a constant fraction, being (0.20) in log units or — 37 % per 2 hrs of exposure to CO.

Series h. In a further series 12 animals were exposed to carbon monoxide of 0.04 % by volume (carboxyhaemoglobin c. 20 %) for four hours, but instead of being subsequently exposed to trichlorethylene without time delay, they were first kept in room

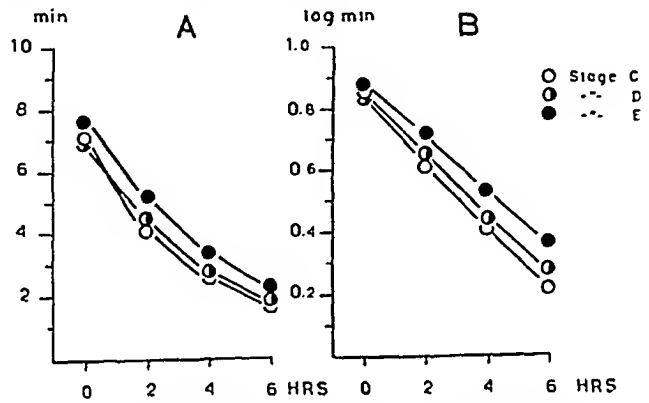

Fig. 5. Induction Times for Stages of Narcosis after Exposure to CO and to Trichlorethylene in Relation to Time of Exposure to CO (0.04 % in Air) (Mean Values).

atmosphere, until the blood was *free* of carbon monoxide, which happened after c. $1^1/_2$ hours, and *then* exposured to trichlorethylene.

The result is given in figure 4.

It is obvious that the exposure for 4 hours to CO caused a definite and significant decrease in induction of all the stages examined, as compared to controls only exposed to trichlorethylene but not to CO. The effect was, however, not so great as after exposure to CO for four hours without the subsequent exposition to air, the times being about those of 4 hours exposure to CO for stages A and B, and close to those of 2 hours exposure to CO for stages C, D and E. Thus in this case stages A and B showed a higher decrease than the stages C, D and E, whereas for all other exposures (series II: e—g) the lowering of the induction time for all stages was a *constant* fraction of those of the controls. One reason for these results being different from those of the other series (II: e—g) may be the influence of exposure to air after carbon monoxide, thus causing an improvement which influences later stages more, a hypothesis, which will have to be elucidated more closely in further experiments.

Series i. Further animals, exposed to 0.04 % CO for 4 hours, were subsequently kept in air for 24 hours, and so exposed to trichlorethylene. No difference from the controls was seen, thus no influence of the CO-exposure on the preceeding day.

III. Short exposure to Carbon Monoxide of Moderate Concentration.

Finally it could also be demonstrated in another way, that this effect of carbon monoxide on induction time did remain for some hours after all the carbon monoxide had been exhaled.

Series j. The animals breathed carbon monoxide 0.2 % by volume in air during 5 minutes that caused about 40 % carboxyhaemoglobin in the blood. If these animals were tested with trichlorethylene immediately, the induction time was the same as that of normal mice unexposed to carbon monoxide.

Series k. The animals breathed CO (0.2 % in air) for 5 minutes, but were then placed in room atmosphere for $1^1/_2$ hour after which their blood was completely free from carbon monoxide, and were then tested with trichlorethylene as usual; the induction time was shorter than in normal animals. This indicates a certain period of time after exposure to be necessary for the development of an influence on trichlorethylene narcosis.

Variability.

The variability between animals for each stage is given in tables 1—3 and is seen to vary between 15.4 and 45.7 % of the average.

Table 1.

Induction Times to Different Stages of Narcosis after Inhalation of Trichlorethylene, 27.9 mg/lit.

Stage	Number of animals	Induction Times Mean ± ε_M		Stand. dev. (σ)		
		log values	minutes	log	Per cent of mean	minutes
A	40	0.519 ± 0.017	3.30 ± 0.13	0.107	24.6 %	0.81
B	39	0.528 ± 0.017	3.38 ± 0.13	0.106	24.4 %	0.83
C	40	0.849 ± 0.012	7.07 ± 0.23	0.077	17.7 %	1.25
D	40	0.836 ± 0.011	6.87 ± 0.17	0.067	15.4 %	1.05
E	40	0.880 ± 0.011	7.59 ± 0.19	0.069	15.9 %	1.21

Table 2.

Induction Times to Different Stages of Narcosis after Exposure to Carbon Monoxide, Preceding Inhalation of Trichlorethylene.

Stage	Number of animals	Induction Times Mean ± ε_M		Stand. dev. (σ)		
		log values	minutes	log	Per cent of mean	Abs. minutes
		0.04 % CO: 2 hrs.				
A	13	0.242 ± 0.040	1.75 ± 0.16	0.146	33.6 %	0.59
B	13	0.285 ± 0.043	1.93 ± 0.19	0.158	36.4 %	0.70
C	13	0.609 ± 0.022	4.07 ± 0.21	0.081	18.6 %	0.76
D	13	0.652 ± 0.019	4.49 ± 0.20	0.070	16.1 %	0.72
E	13	0.718 ± 0.028	5.23 ± 0.34	0.100	23.0 %	1.20
		0.04 % CO: 4 hrs.				
A	12	0.009 ± 0.048	1.02 ± 0.11	0.165	38.0 %	0.39
B	12	0.067 ± 0.057	1.17 ± 0.16	0.199	45.8 %	0.54
C	12	0.405 ± 0.027	2.54 ± 0.16	0.094	21.7 %	0.55
D	12	0.438 ± 0.029	2.74 ± 0.19	0.102	23.4 %	0.84
E	12	0.531 ± 0.023	3.39 ± 0.18	0.079	18.1 %	0.61
		0.04 % CO: 6 hrs.				
A	15	-0.045 ± 0.038	0.90 ± 0.08	0.150	34.5 %	0.31
B	15	0.005 ± 0.031	1.01 ± 0.07	0.119	27.4 %	0.28
C	15	0.223 ± 0.035	1.67 ± 0.13	0.135	31.1 %	0.52
D	15	0.275 ± 0.032	1.88 ± 0.14	0.125	28.8 %	0.54
E	15	0.363 ± 0.027	2.31 ± 0.14	0.104	23.9 %	0.55

Table 3.

Induction Times to Stages of Narcosis after Exposure to 0.04 % CO for 4 Hrs and Air for 1½ Hrs, Preceding Inhalation of Trichlorethylene.

Stage	Number of animals	Induction Times Mean ± ε_M		Stand. Dev. (σ)		
		log values	abs.	log	Per cent of mean	abs.
A	12	-0.065 ± 0.043	0.86 ± 0.09	0.149	34.3 %	0.30
B	12	0.002 ± 0.039	1.00 ± 0.09	0.134	30.8 %	0.31
C	12	0.515 ± 0.026	3.27 ± 0.20	0.090	20.7 %	0.68
D	12	0.628 ± 0.024	4.25 ± 0.24	0.086	19.8 %	0.84

Stages A and B generally seem to have a higher percentual variation, and stages C, D and E have a slighter one. Further the series with exposure to CO (table 2 and 3) show a larger variability than the control series (table 1).

An analysis of the magnitude of the variability in relation to the average discloses that the variation in absolute figures in-

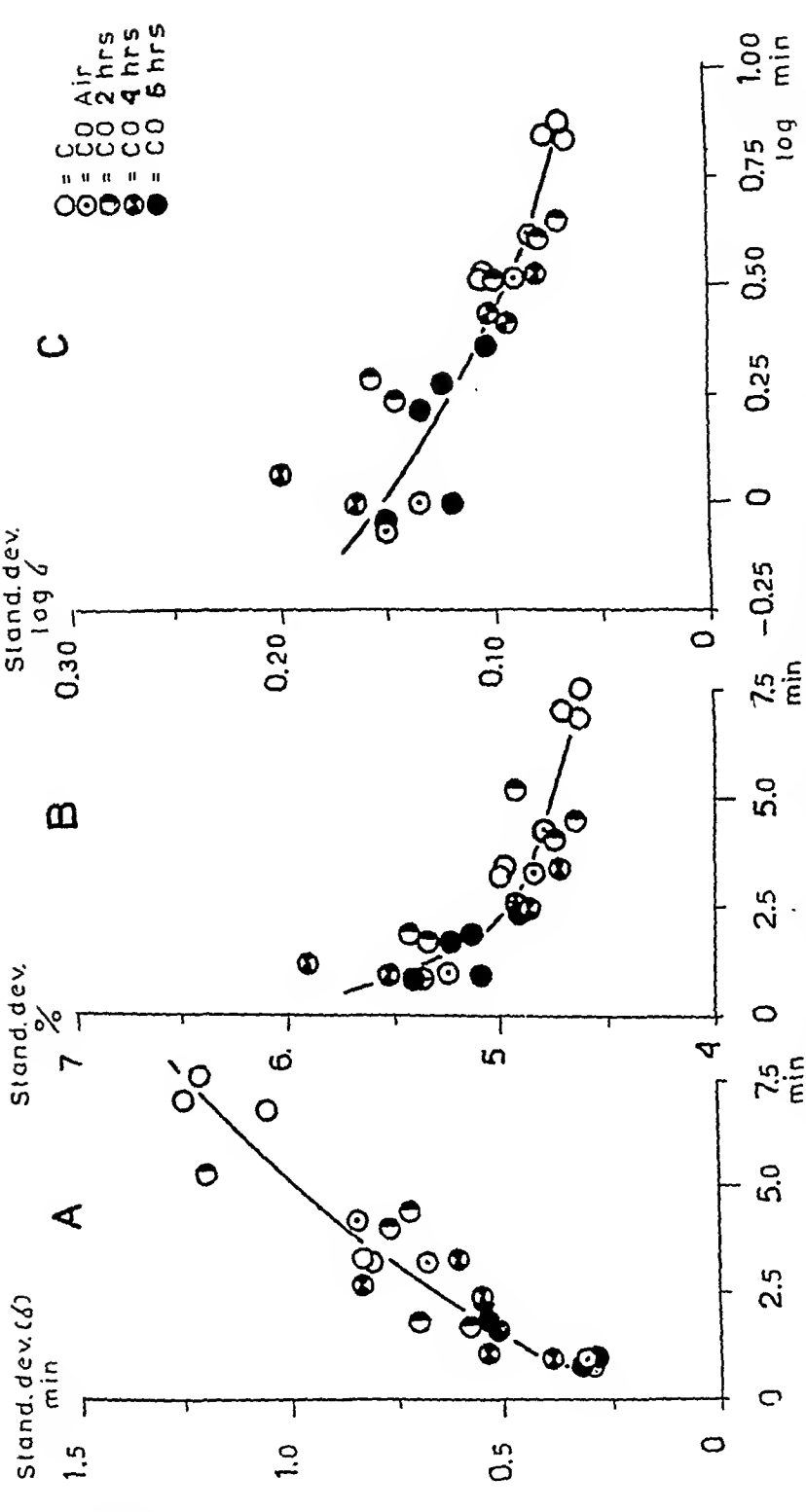

Fig. 6. Standard Deviation (σ) in Relation to Mean Induction Time.

A. σ in minutes in relation to mean in minutes. B. σ in percent of mean in relation to mean in minutes. C. σ from log values (log σ) in relation to mean of log values. 5 series of animals, 85 animals in all.

creases with increasing average (fig. 6 A), the *percentual* standard deviation *decreases* with the average (fig. 6 B), and finally the log stand. dev. decreases only slightly with the log average (fig. 6 C). This implies the stages with longer induction times to have a somewhat larger reliability than that of the earlier stages (A, B), as the percentual variability is slighter with longer duration in spite of the variability in absolute values.

Such a relation between standard deviation and average is also shown for duration of local anesthetics (GOLDBERG 1947).

When comparing the different series performed: control animals (I) and animals, exposed to CO (II) it is obvious that the series with the animals, exposed to CO, show a higher percentual variability.

This may be due to two causes:

a) the shorter induction times after CO-exposure bringing about a higher percentual variability,

b) the exposure to CO causing an increased scatter.

An analysis of the values in figure 6 shows all values to be distributed proportionally to the magnitude of the *average* (see above), but distributed at *random* as to coming from control animals or from animals being exposed to CO. Thus the larger percentual variability of the four CO-series is due to the average induction times being slighter (a), and not to any effect (b), inherent in the action of CO.

Discussion.

A combined action of carbon monoxide and of trichlorethylene, these agents acting synergistically and potentiating each other was obtained by exposing animals to carbon monoxide and trichlorethylene in combination, but only if carbon monoxide was allowed to act on the test animals over a certain period of time.

The results are interesting in illustrating the pharmacodynamics of carbon monoxide. Earlier observers have as a rule used higher concentrations of CO with subsequent unconsciousness, pathologico-anatomical changes in the brain and other signs of a powerful, acute poisoning by carbon monoxide. It has been practically impossible hitherto to study the influence of *low* concentrations of carbon monoxide because of the lack of a suitable method. The determination of time of induction of narcosis after trichlorethylene

exposure, as used here, seems to be a method that can easily be adapted for studying the effect of *low* carbon monoxide concentrations on animals, the change in time of induction, in other words the changed sensitivity to an anesthetic, taken as an indicator of the action of carbon monoxide on the central nervous system.

The findings indicate that the degree of action of carbon monoxide at these low concentrations is not only proportionate to the concentration of carboxyhaemoglobin, but also to the time of exposure. These results of our experiments cannot have been due only to an increase of the carbon monoxide concentration in the blood, anyhow not after 4 hours exposure to CO and subsequent exposure in air for $1^1/_2$ hours', as the CO is very rapidly eliminated in mice with their high respiratory frequency. In our experiments, thus a concentration of carboxyhaemoglobin of c. 18 —28 % does not result in a change of time of induction, when the animals were acutely exposed, but after 2, 4 and 6 hours of exposure there is an increased sensitivity to trichlorethylene as result of the action of carbon monoxide. This is a new "time factor", the existence of which was thus demonstrated by (i) prolonged exposure of animals to carbon monoxide, series II: e—h, and (ii) *waiting* for a certain period of time after *acute* exposure, series II: h, III: k, besides the one earlier known to exist, which determines the equilibrium between blood and air, where prolonged exposure only results in an increase of the carbon monoxide concentration in the blood. Even after the carbon monoxide had been exhaled, and the blood was completely free of it, the effect on time of induction remained for some hours (II: h, III: g), but had completely disappeared the next day (II: i).

The mechanism of this action which, thus, is independent of the momentary concentration of carbon monoxide to a certain extent, is not known.

Cerebral oedema at acute carbon monoxide poisoning has been described (FORBES, COBB and FREMOMT-SMITH 1924), but referred to serious acute poisoning with deep and protracted unconsciousness. In that case the oedema could be observed macroscopically through a cranial window.

No cerebral oedema could be found macroscopically in our experiment animals. Determination of the dry weight of brains from mice exposed to these concentrations of carbon monoxide

for 4—6 hours, gave no statistically proved difference as compared to brains from normal mice. This indicates that cerebral oedema as a possible cause of this late action of carbon monoxide is not probable but cannot be entirely excluded.

Another possible cause may be the fact the intermediary metabolism is inhibited by the anoxia, either because the very inhibition reduces the resistance of the cells to trichlorethylene, or because, at tissue anoxia, normal or abnormal metabolites accumulate in the tissues until the concentration becomes toxic (for literature *vide* FORSSMAN 1941, COLLDAHL 1943).

Experiments are in progress along these lines on the possible relationship between the effects seen and different degrees of anoxia.

Conclusions and Summary.

If mice are exposed to carbon monoxide and trichlorethylene simultaneously, a potentiation of the effect of the two substances is obtained, on the condition that the carbon monoxide is allowed to act for *some time* on the organism. It has been established that a suitable method for studying the action of low concentrations of carbon monoxide in mice is to determine the time of induction for trichlorethylene under certain experimental conditions, and to compare normal animals to others which have been exposed to carbon monoxide.

This was done in 11 series on 277 mice in all, exposed to moderate (0.2 %) and low (0.04 %) concentrations of CO for different times.

The following results were seen:

Mice acutely exposed to CO and immediately exposed to trichlorethylene, show no change in induction time. Mice exposed to carbon monoxide for 2, 4 and 6 hours, until a content of carboxyhaemoglobin of c. 18—28 %, get a shorter time of induction, *i. e.* they become more sensitive to trichlorethylene than normal mice.

This effect remains for some hours after all carbon monoxide has been exhaled and therefore is not only dependent upon the degree of tissue anoxia, but at a constant concentration of carbon monoxide in the blood also upon the time of exposure. The mechanism of this delayed action is discussed.

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Studies on the Initial Changes in Respiration at the Transition from Rest to Work and from Work to Rest.

By

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In previous investigations (ASMUSSEN, CHRISTENSEN and NIELSEN (1943), ASMUSSEN, NIELSEN and WIETH-PEDERSEN (1943), ASMUSSEN and NIELSEN (1946)) it was made probable that the increased ventilation during *light work* is predominantly due to nervous factors, probably proprio-ceptive reflexes from the moving limbs, whereas during *heavy work*, *i. e.* such work in which the muscles are working under partly anaerobic conditions, substances produced in the working muscles account for the further increase in ventilation. The existence of the proprioceptive reflexes from the limbs acting on the respiration during exercise has first been demonstrated by HARRISON, CALHOUN and HARRISON (1932) and recently again by COMROE and SCHMIDT (1943).

BARMAN, MOREIRA and CONSOLAZIO (1943), using the same technique as ASMUSSEN, CHRISTENSEN and NIELSEN, *i. e.* blocking of the circulation to the working muscles by pneumatic cuffs during uninterupted work, conclude from their experiments that chemical factors alone are responsible for the increased ventilation during work, but obviously overlook that whereas the ventilation in their three subjects with blocked circulation decreased only 9, 8 and 7 pCt, respectively, the oxygen uptake decreased 25, 23 and 34 pCt, indicating that factors of nervous origin probably have been active in keeping the ventilation on the high level.

EULER and LILJESTRAND (1946) in experiments with electrical stimulation of the hind-legs of cats and dogs found that the ventilation during the work was increased also after the cord had been severed, and concluded that the increase in ventilation was not due to reflexes but was caused by a direct chemical stimulation of the respiratory centre. Similar experiments have earlier been performed a. o. by KRAMER and GAUER (1941) with the same results, but these authors concluded that the increase in ventilation found during work after cordotomy was caused by the fall in arterial blood pressure that occurs during work under these circumstances. In the experiments of EULER and LILJESTRAND the arterial blood pressure is not listed among their data and the possibility that their results can be explained in the same way cannot be excluded.

HEYMANS, JACOB and LILJESTRAND (1947) could show in experiments with perfused isolated heads of dogs, that when the donor dog was performing electrically induced work the ventilation of the receptor dog increased somewhat. They concluded that this increase in ventilation was due to both an increase in arterial pCO_2 and to a decrease in arterial pO_2. — The fact that an increased arterial pCO_2 or decreased arterial pO_2 under these circumstances can produce a slightly augmented ventilation, however, does not explain the increase in ventilation during work, as the changes found in the chemical composition of the arterial blood of normal men and animals doing moderate work are far too small to account for the observed rise in ventilation.

In the paper mentioned above, EULER and LILJESTRAND also suggest that the increased ventilation in ASMUSSEN, CHRISTENSEN and NIELSEN's experiments with blocked circulation to the working muscles may be caused by sensations of pain in the ischemic muscles. This, however, seems unlikely, first because, as these authors point out, the ventilation in their experiments does not increase with increasing duration of ischemia, secondly because ischemic pains were not always felt even at the end of the blocking period.

Our earlier experiments were made in the steady state of work. In the present experiments we have studied the ventilation at the transition from rest to work and from work to rest under different conditions and repeated some of our previous experiments in steady state by a more detailed procedure.

Methods.

The work was performed on a modified KROGH-bicycle ergometer which allowed the subject to rest and work in the same comfortable reclining position. The ventilation was measured by means of a dry gasmeter and recorded by an inkwriting magneto on the kymograph for every .100 or .200 litres passing through the meter. From this record the ventilation pr. min. could be calculated for any interval of time desired. In the present experiments it was found convenient to calculate the vent/min. for every 0.3 min.

The valves through which the subject breathed had been furnished with a diaphragm devised by VON DÖBELN (1946), by means of which the dead space of the valves is cut down practically to zero. Just outside the expiratory valve a narrow tube is inserted into the expiration tube through which samples of end-expiratory air could be taken during the following inspiration. With tidal airs always above .600 l and no dead space in the valves these samples could be taken as representing alveolar air.

The samples were taken in oiled 10 cc glass syringes previously flushed with alveolar air. A cluster of 5 syringes could be attached to the sampling tube by a small manifold so that 5 samples could be procured at short intervals. The syringes were then removed and a second cluster of 5 attached. It was thus possible to take 10 alveolar samples within 2 minutes. The analyses were carried out on the SCHOLANDER gas apparatus (1947). (For deatils in the use of the syringe sampling tubes with the SCHOLANDER apparatus, see appendix.)

The technique of arresting the circulation to the legs during or after work by pneumatic cuffs was the same as used previously (ASMUSSEN, CHRISTENSEN and NIELSEN (1939)). Electrically induced work was performed by means of the *Myotensor*, as earlier described (ASMUSSEN, NIELSEN and WIETH-PEDERSEN (1943)).

The subjects, young medical students, came to the laboratory in the morning after a light, standard breakfast and rested on the ergometer chair for at least one half hour before the experiments were begun.

Results.

a. *Transition from rest to work.*

On the transition from rest to work the ventilation begins to increase from the very first respiration (KROGH and LINDHARD 1913). Our results conform with this. In fig. 1 are shown the results from 8 experiments on O. W., 4 with a work intensity of 618 mkg/min. and 4 with a work intensity of 1,440 mkg/min. The subject did not know beforehand at which grade of work he was going to work. It will be seen that the increase in ventilation

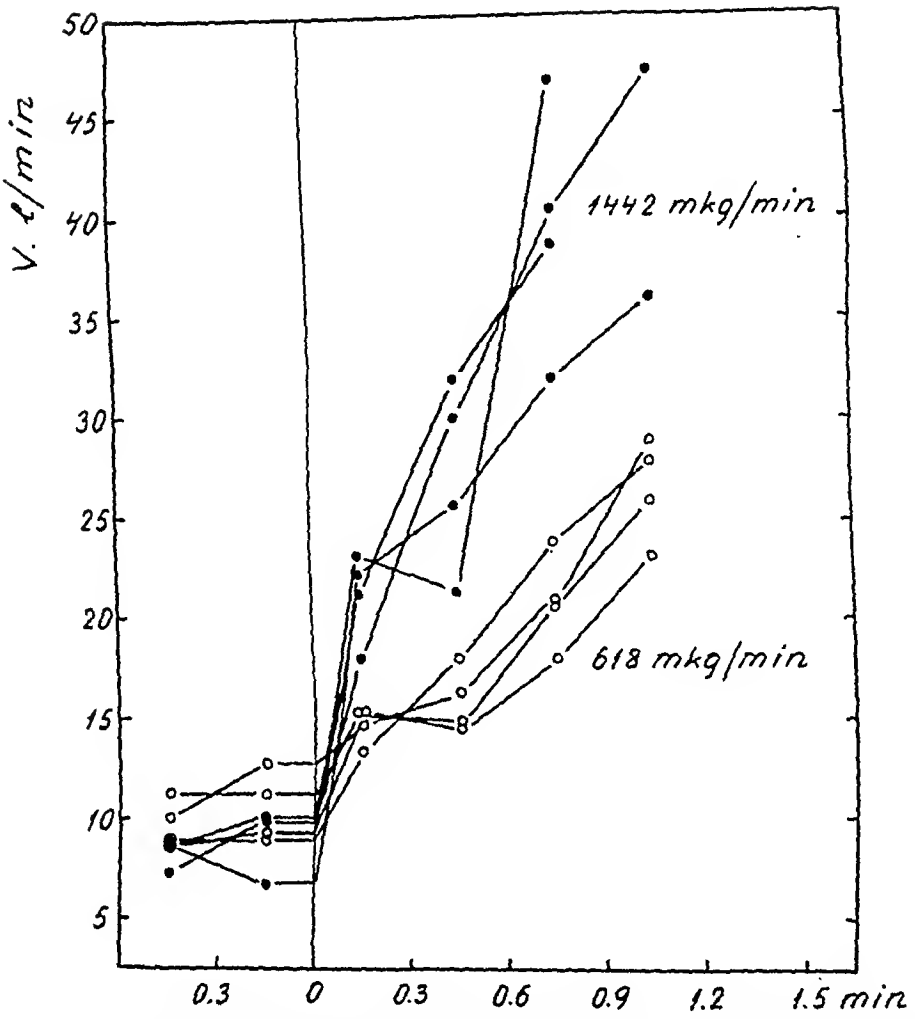

Fig. 1. The initial increase in ventilation during work at 618 mkg/min. (lower curves) and 1,442 mkg/min.]

sets in immediately and that the increase is more rapid in the heavy work than in the lighter work.

In fig. 2 are presented the results from experiments with work varying between 412 mkg/min. and 2,060 mkg/min. The ventilation and the alveolar pCO_2 are plotted against time for the first 0.9 min. of work. The curves show again that the ventilation begins to increase immediately after the start of work and furthermore that the alveolar pCO_2 decreases below the resting value during the first 0.3 to 0.6 min. of work.

In Fig. 3 the ventilation of the first 0.3 min. of work of varying intensity is plotted against the intensity of work. In this fig.

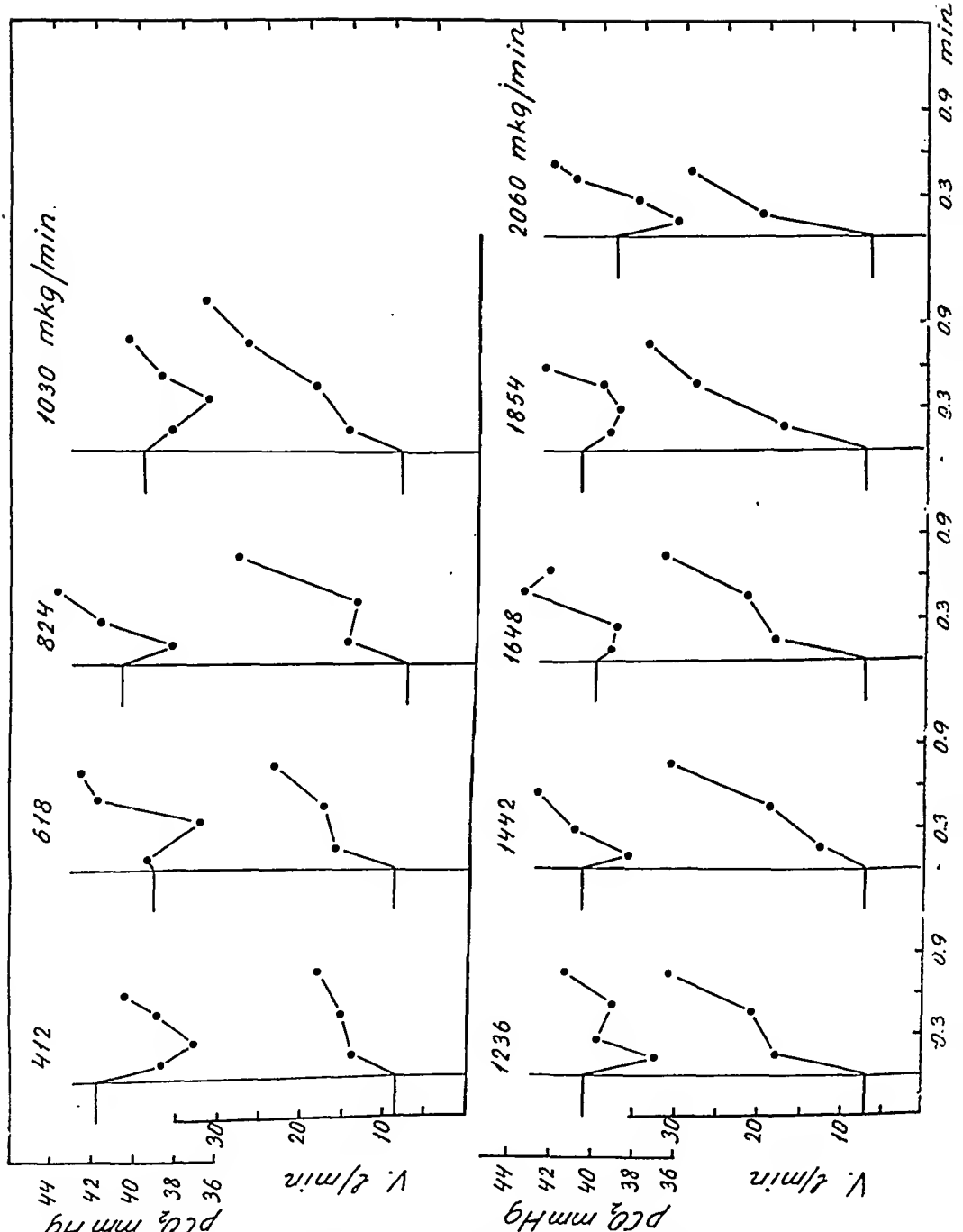

Fig. 2. The ventilation and the alveolar pCO_2 during the first 0.9 min. of work at intensities from 412 mkg/min. to 2,060 mkg/min.

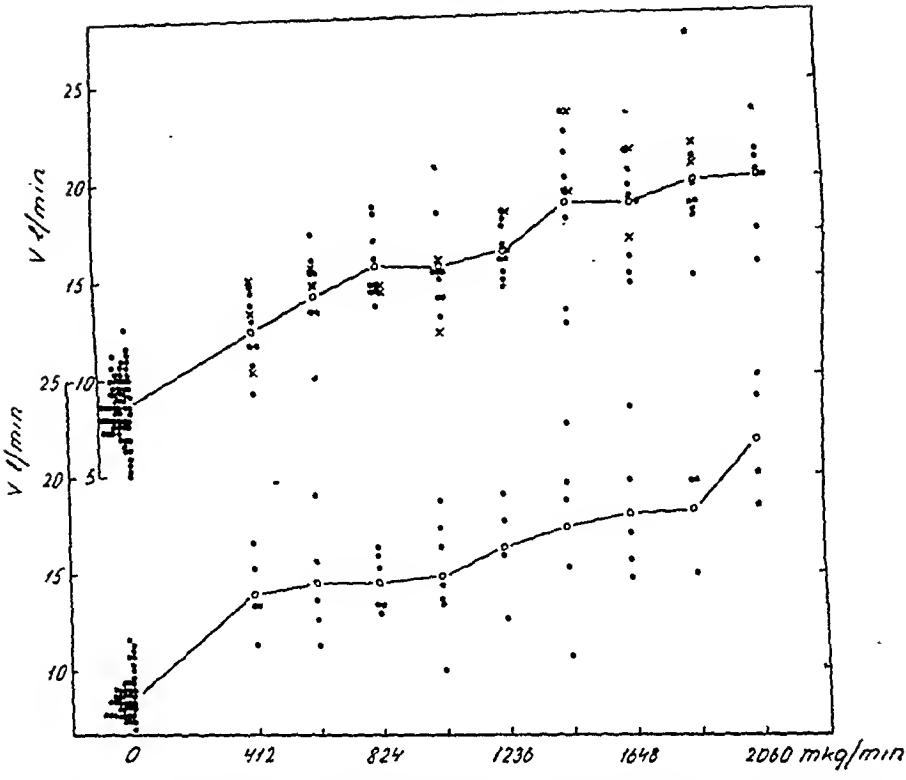

Fig. 3. The ventilation during the first 0.3 min. of work plotted against the intensity of work. Open circles, and lines connecting them represent average values. Crosses represent values from experiments with the circulation to the legs blocked. Upper curve: subj. O. W. Lower curve subj. P. J.

the single determinations are shown as dots. As might be expected, the scattering is rather wide, but the mean values, represented by open circles connected with lines, show that the inititial increase in ventilation is the higher the more severe the work. In both subjects the curve representing the mean values seems to flatten out by increasing work intensity (excepting the one very high value of P. J. at maximum work).

In subject O. W. the circulation to the legs was blocked by pneumatic cuffs round the thighs previous to work in a few experiments. These are shown as crosses in fig. 3 and it can be seen that they lie within the range of the normal experiments.

As mentioned before the subjects did not know beforehand at which intensity of work they were going to work. Pre-knowledge of the severity of the work therefore is not responsible for the normal increase in ventilation. This was further shown in a few mock-experiments, in which the subjects were made to believe that the work they were going to do was hard, whereas

it actually turned out to be work on the unloaded ergometer. In these cases the ventilation for the first 0.3 min. of work in both subjects was but slightly higher than the resting ventilation.

An elimination of the cortical influences on the increase in ventilation was attempted in experiments with electrically in-

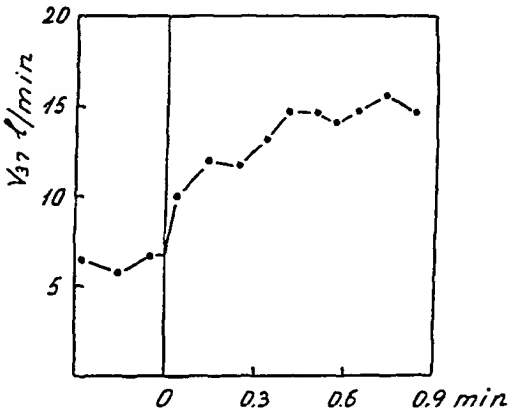

Fig. 4. The initial increase in ventilation during electrically induced work.

duced work. The results of such an experiment is shown in fig. 4. It will be seen that also in this case the ventilation begins to increase immediately, that is before metabolites can have reached the respiratory centre from the working muscles.

b. *Steady state.*

If during the steady state of work the circulation to the legs is suddenly blocked by pneumatic cuffs, the oxygen uptake and CO_2 output through the lungs decreases sharply, whereas the ventilation continues on the same level as before the circulatory arrest. This has been shown earlier by ASMUSSEN, CHRISTENSEN and NIELSEN (1943) and it is demonstrated again in more detail by fig. 5.

Fig. 5 shows two sets of experiments, one with a work intensity of 412 mkg/min., one with 824 mkg/min. The period of occlusion lasted in both cases 2.1 min. It is evident that the ventilation, despite the considerable decrease in oxygen-uptake (in other experiments determined to be about 40—50 pCt of the extra working O_2-uptake) remains practically constant. For the lighter work the alveolar pCO_2 is shown to decrease about 4 mm, for the heavier work (only 4 single determinations, not shown on the curve) the decrease was about 7 mm. When the pressure in the

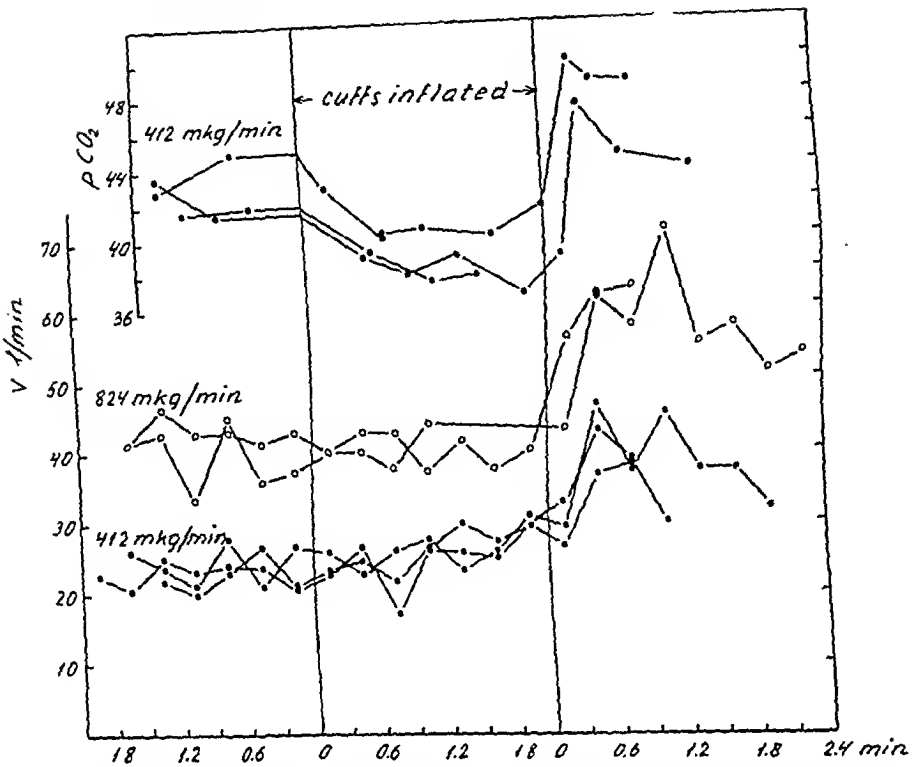

Fig. 5. Ventilation (two lower sets of curves) and alveolar pCO_2, in work before, during and after blocking of the circulation to the legs by means of blood pressure cuffs. The curve showing the alveolar pCO_2 is from the work experiments at 412 mkg/min.

cuffs is released, the alveolar pCO_2 and the ventilation increase markedly after a short delay, owing to the time it takes for the accumulated metabolies to reach the lungs and the centres.

In the experiments here described there were no ischemic pains during the period of occlusion but only a growing feeling of numbness and an increasing difficulty in moving the legs. There is, therefore, hardly any doubt that the cortical motoric innervation of the ischemic muscles must have been considerably augmented in order to keep the output of work constant.

In experiments with electrically induced work the stimulation of the muscles could be kept constant during the circulatory block. Fig. 6 shows the result from such an experiment. It will be seen that the mechanical work in this case decreases gradually as fatigue of the muscles sets in and that the ventilation decreases correspondingly. There were no pains at all in this kind of experiment, the muscles simply diminished their contractions in spite of the continued electrical stimulation.

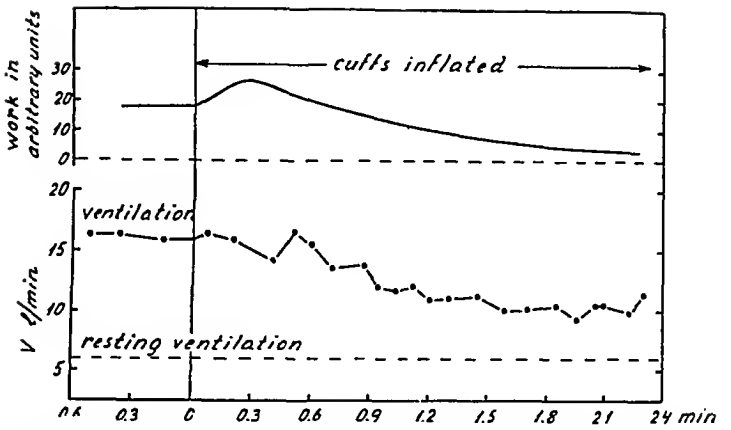

Fig. 6. Ventilation during electrically induced work before and during blocking of the circulation to the working muscles by means of blood pressure cuffs. Upper curve represents the work in arbitrary units.

c. *Transition from work to rest.*

When work stops a decrease in the respiratory functions sets in. In fig. 7 are presented the ventilations from 2 experiments with 412 mkg/min., 3 with 824 mkg/min. and 2 with 1,236 mkg/min. It is seen, that the ventilation begins to decrease immediately after cessation of work, *i. e.* before any change in the blood irrigating the respiratory centre or the chemoreceptors can have occurred. The upper set of curves in fig. 7, shows that the alveolar pCO_2 after work of 824 mkg/min. is practically constant during the first 0.6 min. of recovery, *i. e.* during a time in which the ventilation is markedly decreasing. After this the pCO_2 decreases and falls to about 35 mm Hg in these experiments. In experiments with heavier work the decrease was still more pronounced. After 1 or 2 minutes the normal values of around 40 mm Hg are again approached.

If pneumatic cuffs around the thighs are inflated shortly before stop and are kept inflated during the first minutes after stop, the ventilation will behave as in fig. 8. In this fig. two curves are shown, depicting the events after work of 412 mkg/min. and 824 mkg/min., respectively. It will be noticed that the ventilation also in this case falls sharply at cessation of work, approaching the normal resting value. If anything, the decrease in these experiments is more rapid than in normal conditions, owing to the fact that the alveolar pCO_2 is lowered in the period of occlusion (comp. fig. 5). On release of the pressure the alv. pCO_2 and the ventilation as usually show a passing increment.

Discussion.

The results of the present experiments show, that in the initial phase of work the ventilation increases before any metabolites from the working muscles can reach the respiratory centre. The alveolar pCO_2 will consequently be lowered as in hyper-

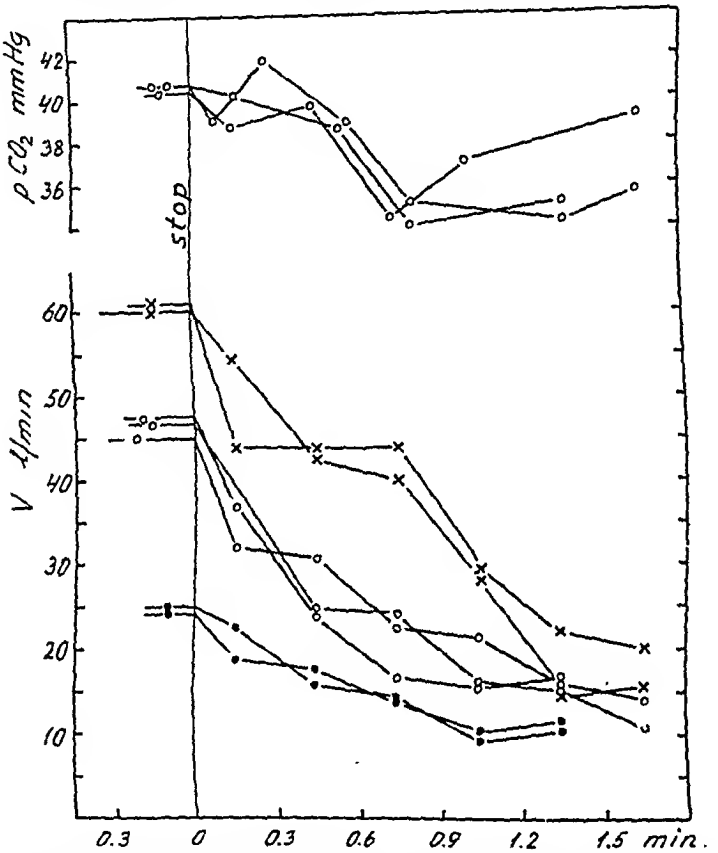

Fig. 7. The ventilation and the alveolar pCO_2 (upper curve) immediately after cessation of work.

●—● 412 mkg/min.
○—○ 824 mkg/min.
×—× 1,236 mkg/min.

ventilation (fig. 2). This is in complete agreement with the results of KROGH and LINDHARD (1913, 1917), and we also agree with these authors in concluding that a nervous regulation must exist.

KROGH and LINDHARD suggested irradiation of the cortical motoric impulses to the respiratory centre as the most likely in voluntary work. The experiments with electrically induced work (fig. 4), however, show in accordance with earlier results

from the steady state of work (ASMUSSEN, NIELSEN and WIETH-PEDERSEN (1943)) that cortical impulses are not necessary for the increase in ventilation during work, but that other nervous impulses, probably from the proprio-ceptors of the moving limbs, play a more important rôle.

The curves of fig. 3 show that the initial reflex increase in ventilation is correlated to the rate of work. It seems natural to assume that the proprio-ceptive impulses from the working legs

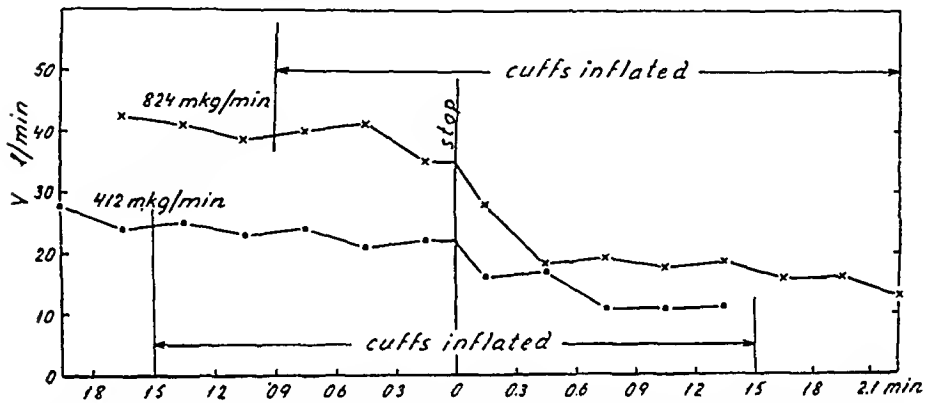

Fig. 8. The ventilation during and after work at 412 mkg/min. and 824 mkg/min. The circulation to the legs blocked as indicated by means of blood pressure cuffs.

are in some way or other integrated so as to produce an effect that is proportional to the mechanical tensions developed during the work. It might be supposed that with increasing intensity of work increasing amounts of blood from the working muscles may reach the respiratory centre or the chemoreceptors, even in the short time of 0.3 min. and that this were responsible for the correlation between the work intensity and the ventilation in fig. 3. Against this assumption speaks the fact that the curves are flattening out at the heavier work intensities. This is, however, in good accordance with the observation made earlier (ASMUSSEN and NIELSEN (1946)) that the reflex impulses from the limbs are able to increase the ventilation only up to a certain point beyond which a new factor of chemical nature is responsible for the further increase in ventilation.

The results from the experiments in steady state (fig. 5 and 8) confirm the experiments of ASMUSSEN, CHRISTENSEN and NIELSEN (1943) that blocking of the circualtion to the legs causes a marked decrease in oxygen uptake and CO_2-output, without a corresponding decrease in ventilation. In some cases, as in fig. 8, the decrease in pCO_2 manifests itself by a slightly decreasing

ventilation (as in the experiments of BARMAN, MOREIRA and CONSOLAZIO (1943)), in other cases the ventilation shows a slight tendency to increase (fig. 5, light work). This slight increase, most pronounced towards the end of the occlusion period, is probably connected with the increasing difficulty in keeping the work constant. (Pains were not experienced by the subjects in these experiments.)

As the anaerobic conditions in the blocked muscles grow more pronounced it is necessary to innervate more motor units in the working muscles in order to keep the rate of work constant. The cortical activity, consequently, must be assumed to be steadily increasing. The negligible effect this has on the ventilation indicates that cortical factors, *e. g.* irradiation of the motor impulses, are of minor importance for the regulation of the ventilation in the steady state of light work, as concluded earlier by ASMUSSEN, NIELSEN and WIETH-PEDERSEN (1943) from experiments with electrically induced work and normal voluntary work. When on the other hand the impulses to the working muscles are kept at a constant strength — as in the electrically induced work — the work performed and the tensions set up by the muscles during the period of circulatory occlusion will decrease steadily, and in parallel to this the ventilation diminishes (fig. 6).

By the transition from work to rest the decrease in ventilation begins without simultaneous changes in the alveolar pCO_2. Apparently at the stopping of the movements some factor which has helped in keeping the ventilation adjusted to work is diminished, and the rapidity with which this happens shows that it must have been of nervous origin; in consequence of the discussion above it is fair to conclude that it must have been the reflexes from the moving limbs.

As the ventilation, however, for some time still remains increased and only after several minutes returns to the resting level, in spite of the fact that the chemical stimulus (the alveolar pCO_2) is not increased, shows that the excitability of the respiratory centre only gradually returns to its normal resting value. For a certain period the ventilation is so high that the alveolar pCO_2 even undergoes a decrease. (The decrease in alveolar pCO_2 in this period is due to a washing out of CO_2 as indicated by an increased R. Q.)

After the *heavy* work the gradual fall in excitability is no doubt due not only to the gradual diminishing of the effect of

the nervous factors but also to a gradual decrease in the concentration of anaerobic metabolites.

It has been suggested that local chemo-receptors in the muscles might be responsible for the sensoric impulses acting on the respiration, mostly on account of the results of ALAM and SMIRK (1937). These authors claim to have shown that the arterial blood pressure remains high after cessation of work if the metabolites produced in the working muscles are prevented from disappearing from the muscles by inflated pneumatic cuffs. We have tried unsuccessfully to reproduce ALAM and SMIRK's experiments. For the regulation of the ventilation, fig. 8 shows, that the ventilation decreases towards the resting level at the same rate as in normal conditions although large amounts of muscle metabolites are trapped in the legs. The reflexes consequently cannot be chemically evoked but must as previously suggested be proprioceptive.

Summary.

At the *start of work* it is found in conformity with earlier results of KROGH and LINDHARD (1913, 1917) that the ventilation increases so rapidly that only a nervous regulation can be responsible. In electrically induced work the increase begins just as soon as in normal work. The increased ventilation in the first 0.3 min. of work is correlated to the severity of work.

In the *steady state of light work* (voluntary or electrically induced) the ventilation is closely related to the intensity of work, even when the circulatory connections to the rest of the body are blocked by pressure cuffs.

In the *transition from work to rest* the ventilation begins to decrease immediately, before changes in the arterial blood can have occurred. These experimental results add further evidence to the assumption that reflexes from the working limbs play an important part in the increase of ventilation during light work.

Appendix.

On the use of syringe gas sampling tubes in connection with the SCHOLANDER gas analysis apparatus.

Ordinarily, gas samples are stored over mercury. Besides undeniable advantages the mercury filled sampling tubes have

Table I.

0	after 3—4 hours	after 5—6 hours	after 24 hours
3.09 (2) 18.17	3.07 (2) 18.12	3.08 (2) 18.15	
3.58 (4) 17.61	3.59 (2) 17.64	3.58 (2) 17.59	3.51 (2) 17.58
3.15 (3) 18.30	3.16 (3) 18.25	3.17 (2) 18.27	3.07 (3) 18.24
3.31 (4) 18.06	3.30 (1) 18.06	3.29 (1) 18.00	
3.25 (2) 17.32	3.21 (5) 17.31	3.25 (3) 17.28	
4.79 (1) 15.87	4.73 (4) 15.86	4.75 (2) 15.83	
3.73 (2) 17.25	3.74 (2) 17.22		3.70 (1) 17.23
3.09 (2) 18.19		3.07 (2) 18.15	
3.18 (3) 17.93	3.19 (2) 17.94	3.21 (2) 17.93	
3.51 (2) 17.82			3.31 (3) 17.83
3.18 (3) 17.74	3.21 (3) 17.72	3.15 (3) 17.69	
3.16 (4) 17.68		3.11 (4) 17.64	

First column are analyses made immediately after sampling. Each pair of figures gives CO_2 and O_2 percent of sample. Figures in brackets indicate number of single analyses.

certain drawbacks: they are heavy, unwieldy, the mercury spills easily and is expensive and poisonous. Gas sampling without mercury has been used *e. g.* in CARPENTER'S laboratory, and recently, on the suggestion of Dr. DILL, 50 cc oiled glass syringes were tested at the Harvard Fatigue Lab. as sampling tubes (by F. CONSOLAZIO and one of the authors). These tests fell out satisfactorily *i. e.* gas samples remained unaltered for at least 24 hours (not published results). With the introduction of the SCHOLANDER gas analysis apparatus much smaller samples (0.5 cc) can be used for an accurate analysis, and consequently 10 cc glass syringes were tested in this laboratory as sampling tubes for ordinary respiratory gases.

Ordinary all glass 10 cc syringes were greased with enough paraffin oil to make them air tight for light pressure and suction. On the tip of the syringe about 3 cm of thick walled, 1 mm bore

rubber tubing was placed (fig. 1). The rubber tubing could be compressed by an ordinary screw clamp. Expiratory air was sucked into a series of syringes, some of which were analysed at once, others after several hours. These latter were kept in a beaker in an inverted position, resting on the knob of the plunger, so that a slight overpressure was achieved. The table shows the result of some such experiments.

It seems justifiable to conclude that samples keep satisfactorily constant during 6 hours, but that in 24 hour some CO_2 seems to

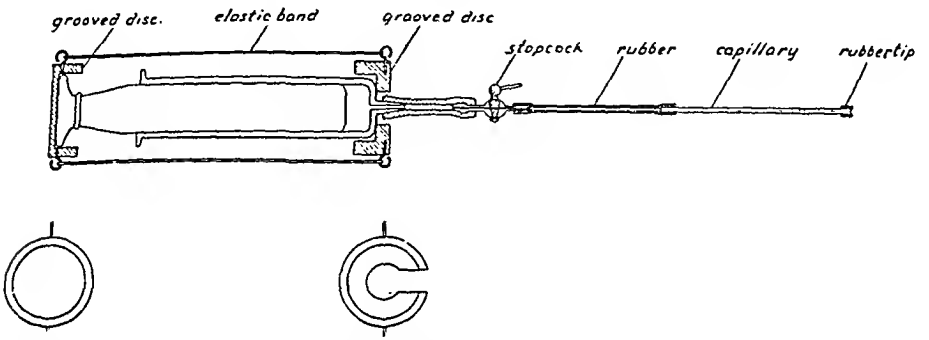

Fig. 1. Syringe sampling tube with transfer-tube. Below the hard rubber discs, one with a slit, are shown.

escape. The mean decrease in the CO_2 per cent after 3—4 hours is 0.007, after 5—6 hours 0.012 and after 24 hours 0.095. The corresponding values for O_2 are: after 3—4 hours 0.014, after 5—6 hours 0.034 and after 24 hours 0.025. For ordinary metabolic studies the small changes that occur within the first 6 hours of storage are negligible.

The advantages of using 10 cc glass syringes for air sampling lies in their cheapness, smallness and lightness, and in the fact, that mercury can be avoided in their handling.

We use them with the SCHOLANDER apparatus in the following way: On the knob and basis of the air filled, closed syringe are placed grooved hard rubber discs, the lower one with a hole and a slit, connected by two elastic rubber bands (fig. 1). A transfer-tube, consisting of a small brass stop cock (bore 0.7 mm), a thin-walled capillary rubber tube, 5 cm long, and a piece of drawn glass capillary (about 3 cm) furnished with a small rubber tip, is connected with the piece of rubber tubing on the syringe. The stop-cock is opened, and then the screw clamp is removed. The overpressure, set up by the two rubber bands will force air out

from the syringe and the plunger will move downwards, braked by the resistance in the transfer-tube. When 3—4 cc of air have disappeared, the dead space of rubber tubing and transfer tube is considered adequately flushed, the stop-cock is closed and the syringe attached to a clamp above the SCHOLANDER-apparatus. The air is now taken into the apparatus by placing the rubber tip of the transfer-tube on the inlet of the SCHOLANDER-apparatus, opening the stop-cock and letting the rubber bands produce the necessary overpressure. As only about 0.5 cc of air is necessary for an analysis, the syringe will still hold plenty for check analysis.

The analysis proceeds in the way described by SCHOLANDER with the exception that we have found it unnecessary to close the compensating chamber during the analysis; consequently we have omitted the ground glass stop-cock and thus simplified the otherwise very simple procedure still more. The analytical accuracy was found as stated by SCHOLANDER, *i. e.* the same as on the HALDANE-apparatus.

The hard-rubber discs and transfer-tubes with stop-cocks were made by the mechanic of the laboratory, Mr. CHR. PERSSON. He also makes the micrometer-screw of the SCHOLANDER-apparatus, the glass parts of which are made by Dansk Glasapparatur c/o Mr. ANG. JENSEN, Copenhagen.

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From the Department of Anatomy, Aarhus University, Denmark.

Phosphatase in Dogs Following Bile Obstruction and Removal of the Small Intestine.

By

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Received 24 May 1948.

Obstruction of the common bile duct in dogs is followed by an increase in alkaline serum phosphatase. This has been shown by many authors and through previous experiments of the present writer.

Some facts point towards the intestine as the source of this hyper-phosphataseaemia. That is, in the first place, the normal high content of phosphatase in the intestinal mucosa, which seems to exeed the content of all other organs. ARMSTRONG and BANTING 1935, OPPENHEIMER 1935, FOLLEY and KAY 1936, GAD 1946, HOFFMEYER, JALLING and SCHØNHEYDER 1946.) These findings are in conjunction with the findings by means of the histochemical method, which reveals a very high concentration of phosphatase especially in the duodenal and jejunal epithelial cells.

Secondly, the serum phosphatase level is influenced through the diet (References by WACHSTEIN 1945) especially in the case of rats where the dietary factor is far more important than the obstruction of the bile duct (WEIL and RUSSEL 1940 and 1942, DALGAARD 1947): This likewise points towards the intestine.

Lastly, however, should be mentioned the interesting results of GAD 1946: that during obstructive jaundice in dogs the amount of phosphatase in the jejunal mucosa increased to three times the normal amount. This finding led GAD to assume that the increased serum phosphatase originates from the intestine. (Comp.

HOFFMEYER, JALLING and SCHØNHEYDER, 1946, who did not find such an increase in rabbits' intestines.)

This paper does not intend to discuss the phosphatase problems during obstructive jaundice in general; but only to evaluate the rôle of the small intestine through surgical experiments in which the duodenum or the complete small intestine is removed, the common bile duct ligated, and the serum phosphatase examined.

Such experiments have not been performed previously. Here only should ne mentioned the experiments of ARMSTRONG and BANTING 1935, in which various organs were systematically removed in dogs whose common bile duct was *not* ligated. In the experiments with removal of the intestine, three inches of the duodenum was left and anastomosed to the terminal inch of the ileum. As no decrease in phosphatase occured it was concluded that neither the intestine nor any one of the other organs removed was the source of the normal serum phosphatase.

Experimental.

Material and methods. Eleven adult dogs were used. Previous to the operations they were fed a liberal diet of milk and rye-bread with margarine. Concurrently the phosphatase was estimated 2—4 times in each animal. The analyses were done by means of the BUCH and BUCH method (1939), but all reagents were diminished to $^1/_5$, *i. e.* 0.1 ml of serum was used. (Comp. DALGAARD 1948.)

P r e o p e r a t i v e l y, after 12 hours fasting, morphine (0.4 cg/kg), eventually supplemented with scopolamine or intravenously hypnophene (1—2 ml), was given. Ether narcosis was used, except in H_{18}, $_{19}$ and $_{34}$, where narconumal Roche was given intravenously. In a control animal it was proved that such medication and narcosis was without influence on the phosphatase.

Removal of the duodenum and pylorus was done in two dogs in which gastro-jejunal anastomosis was made 14 days previously. T e c h n i q u e: Through a para-median section the duodenum was divided just above the duodeno-jejunal flexure. The jejunum was invaginated while the duodenum and pyloric portion of the stomach was carefully freed from the pancreas and removed, all vessels, the pancreatic duct and the common bile duct being minutely ligated. After exploring the anastomosis made at the previous operation, the stomach was closed. Postoperatively subcutaneous saline solution was supplied.

Result: The anastomosis-operation did not influence the serum phosphatase. H_{13} died of peritonitis on the third day and is therefore omitted. (There was no noteworthy increase in the phospha-

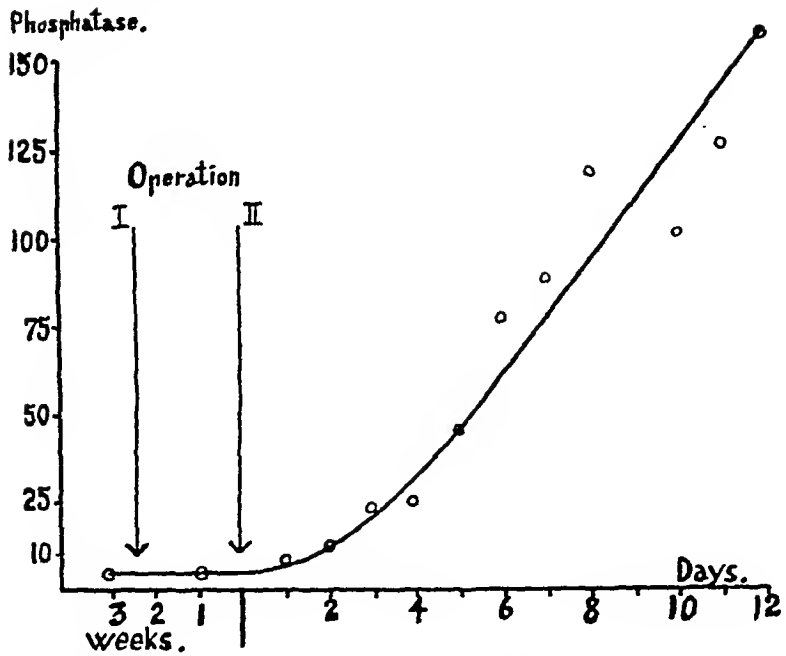

Fig. 1. Phosphatase curve from H_8 in which a gastro-jejunal anastomosis was made at a primary operation (I) without influence on the serum phosphatase. 3 weeks later (at II) the duodenum and pyloric part of the stomach was removed and the common bile duct ligated. This was followed by a very pronounced increase in serum phosphatase.

tase). H_8, on the contrary, displayed an appreciable increase in serum phosphatase, detectable already the day after the operation, reaching double the normal value the third day and surpassing eighteen times the normal value on the 12th day. See fig. 1.

Total removal of the small intestine, with ligation of the common bile duct was done in 8 dogs. Technique: Through a median section the ileum was divided 2—3 cm from its termination and invaginated into the coecum. In 4 dogs (H_{31-35}) a rubber tube was inserted into the coecum and drawn outside, thus constituting a coecal-fistula for supplying glucose solution. The mesenteric vessels were ligated and the small intestine removed, leaving in dogs $H_{14, 18, 19}$ and $_{22}$ about $1^1/_2$ cm of the duodenum, which was crushed and invaginated. In the other 4 dogs the whole of the duodenum and the pyloric portion of the stomach (about 5 cm) was removed with the intestine as described above.

After-care is very important after the extensive operations. Furthermore, dehydration in itself would cause an elevation in

Table.

In the table the individual serum phosphatase estimations are given from 7 dogs, before and after removal of the small intestine with simultaneous ligation of the common bile duct.

No.	Days before operation			Days after operation.						
	6—3	2—1	0	1	2	3	4	5	6	7
18	6.5	6.5	3.9	110	210					
14	3.7	5.5	5.5	26	26	81	81			
19		8.4	10	21 23						
22	3.7	2.6		10	14 18	36	100	104		
31		0.8	3.7	8.4 13.4						
33	5.8	7.4		32						
35		1.1	3.4	15	45		79 74	84	74	84
Mean:		4.9								

the serum phosphatase. Suitable surrounding temperature (25° C.) was attained and liquids and calories were supplied (saline subcutaneously, glucose solution and amino acids intravenously and through the coecal-fistula or rectum). Loss in weight could not be quite prevented, however. Occasionally plasma bicarbonate and chloride was determined, without showing notable deviations.

Results: H_{34} died within 24 hours and is omitted from the table. Four animals ($H_{18, 19, 31}$ and $_{33}$) died 40—48 hours after the operation, but all showed increase in phosphatase. In H_{18} liver-infarcts caused death after 36 hours. In this dog the maximal increase in phosphatase was observed, but because of the liver-disorder this animal could not be regarded as a "pure" case. In H_{19} lung-infarcts caused the death, but in no other case infarcts were observed at the autopsy. $H_{14, 35}$ and $_{22}$ survived for 4, 8 and 7 days, respectively. The serum icterus index was increased and the increase in phosphatase was very pronounced. Se the table and the curves in fig. 2.

Total removal of small intestine 4 days after common bile duct ligation was undertaken in one dog. The maximal values of serum phosphatase, caused by the ligation, were *not* diminished through removal of the intestine. The dog survived for four days after the second operation.

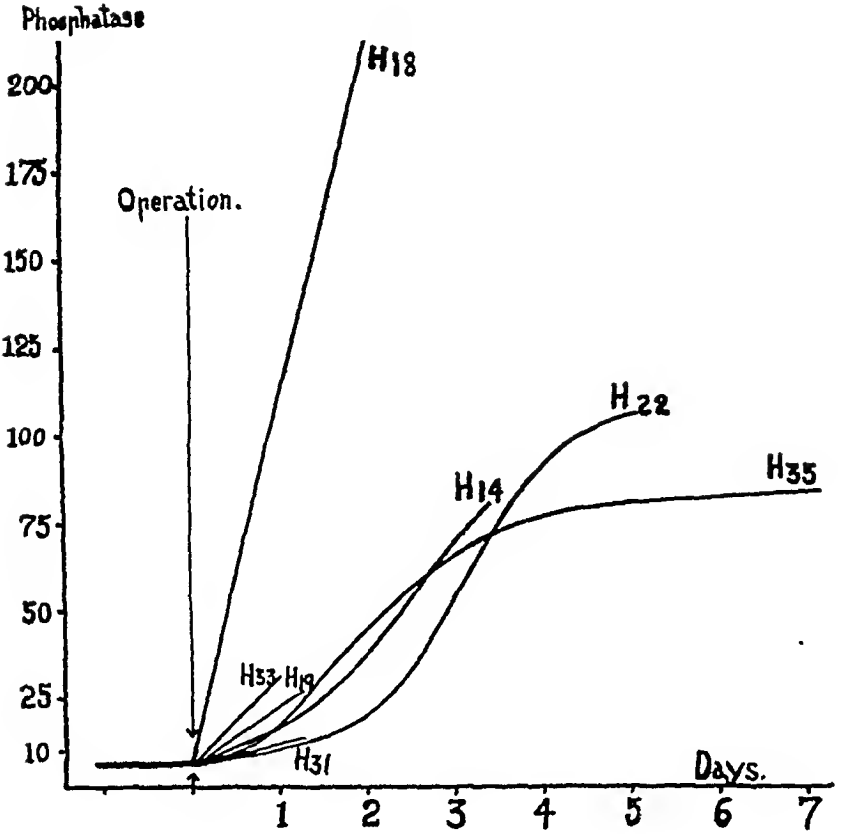

Fig. 2. Phosphatase curves from 7 dogs in which the small intestine was completely removed, and the common bile duct ligated. In all animals an increase in serum phosphatase was observed, which, in the dogs surviving more than two days, reached very high levels.

Summary.

In spite of the complications which developed in some of the dogs after the extensive operations, it has been proved that neither the removal of the duodenum-pylorus nor of the total small intestine prevents the increase in phosphatase which always occurs following ligation of the common bile duct.

The intestine is thus excluded as the source of the increased phosphatase.

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Serum and Bile Phosphatase in Biliary Fistula Dogs.

By

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Received 6 September 1948.

To explain the increased serum phosphatase during obstructive jaundice it is important to study the phosphatase even in cases of biliary fistula. As previous investigations in this field have yielded rather astonishing and somewhat differing results, which might be attributed to the technical difficulties in maintaining functioning biliary fistulas, it seems important to revise the problem considering especially the technique and the criterions for the sufficiency of the fistulas.

A Critical Survey of the Results of Previous Authors.

For practical reasons the communications on clinical cases, rabbits and dogs shall be considered separately.

Clinical cases: AUSTONI and COGGI (1934) gave the first report on the problem and compared it with the findings during bile obstruction: "Dans les dérivations biliaires externes, totales et partielles, existant depuis plus q'un mois, la phosphatase a présenté une augmentation moins considérable, mais toujours évidente et constante."

MORRIS and PEDEN (1937) examined the phosphatase "at weekly intervals in a patient with an external biliary fistula. During the period when no bile was entering the gut, the plasma phosphatase increased steadily to about four times the normal value although there was no jaundice."

C o m m e n t s: Both communications are very brief. Only the context of the first quotation indicates that humans seem to be concerned. And the term "partial or total fistula" proves the uncertainty of the statements. The second quotation also most probably concerns a patient with bile duct stone causing transient obstruction.

The evaluation of clinical cases is always difficult, especially when autopsy is not performed. The fistula cases quoted here, at any rate do not seem to have been uncomplicated ones, and in the author's opinion too much importance has been attributed to them.

Bile fistulas in rabbits: IBSEN (1945) did not observe increased phosphatase in his three bile-fistula rabbits, but as they were analysed only 2—4 hours after operation the results are of little significance.

JALLING, LAURSEN and VOLQVARTZ (1945), introduced a rubber drainage tube into the gall-bladder in three rabbits and ligated the common bile duct. Phosphatase was estimated in the bile during the first 24 hours and in the serum during three days. T h e s e r u m p h o s p h a t a s e d i d n o t i n c r e a s e!

The phosphatase excreted in the bile during the 24 hour period was of small amount only, and even a complete retention thereof would have been insufficient to explain the increase in serum phosphatase which occurs in rabbits with ligated common bile duct.

C o m m e n t s: Although gall-bladder fistulas, at least in dogs, cannot be regarded as the most serviceable (see below), these experiments have been successful and their main result is very important. Unfortunately the observation times are very short and the fate of the animals is not stated, probably they died when the observations ended. But the writer will definitely warn against any conclusion drawn from the observations in bile during the first 24 hours, as the amount of bile during this time is always exceedingly small (see later) and the phosphatase values thus will be much too small.

Bile fistulas in dogs: THANNHAUSER et al. (1937) gave the first report on phosphatase in bile fistula dogs, 12 in all, in most of which a catheter was introduced into the gall-bladder, while in a few (unstated number) the ROUS-MCMASTER technique was used. Increases in phosphatase to 20 times the normal value were reported, with a maximum about the 7th day, after which a decrease occurred. The bile was collected but the amounts not stated.

Comments: Unfortunately the phosphatase values were given for two dogs only, possibly those showing the maximal increase. It is not mentioned whether all dogs displayed increase. Statements about the amounts of bile, icterus index and autopsy are entirely missing. These serious omissions leave the reader uncertain whether the fistulas have really been properly functioning, and the results are thus, in fact, incapable of being judged.

FREEMAN, CHEN and IVY (1938) performed 12 estimations of serum phosphatase in 9 different dogs with bile fistulas and observed an increase which was often appreciable. The amount of the bile was not measured, nor was autopsy performed.

Comments: The experimental results are fully elucidated in the paper. Here shall be stressed very strongly, that all real high phosphatase values occurred in dogs with icterus or infection or both. According to the requirements given below for regarding fistulas as sufficient, only 4 values can be considered, and they all indicate a quite modest increase, if any at all.

BALCELLS-GORINA and SOLS (1946) observed a transient increase in phosphatase in a single dog with *partial* fistula — which in fact also means partial obstruction.

DRILL, ANNEGERS, SNAPP and IVY (1945) studied 14 dogs with bile fistulas (a rubber tube through the cystic duct into the common bile duct). Serum phosphatase, bile volume and bile phosphatase and bromsulphonphthalein-retention (br.-ret.) was estimated.

Most animals sooner or later displayed increase in serum phosphatase and br.-ret., mostly concurrently. In two dogs the phosphatase increase was only transient, in 5 dogs the increase did not occur until 8—10 days after the operation, and 3 dogs hardly displayed any increase at all. It was stated that the amount of bile was normal. The authors analysed the results, and supposed that the phosphatase excretion through the bile sometimes could compensate an increase in the serum, sometimes not.

In 6 further dogs "internal fistulas" were produced through anastomosing the gall-bladder with the renal pelvis, while the common bile duct was ligated. In 5 animals jaundice and increased br.-ret. occurred. In these cases the phosphatase was increased, in one dog, however, only slightly and transiently and in another one not until 3 weeks after the operation. The 6th dog did not become jaundiced and in this one both br.-ret. and phosphatase were normal.

Comments: Many of the experiments lasted 2 months, and it should be stressed that the increases in phosphatase often occurred late, while the phosphatase level was often rather low during the first week or more. The discrepancy with the results of the present author is thus less than supposed according to a superficial comparison.

The phosphatase increase observed by the authors is, at any rate, much less than the one observed during bile duct obstruction. This is clearly demonstrated in a dog in which obstruction occurred, after which the phosphatase increased markedly.

Scott (1945) whose 4 internal bile fistula dogs, fed milk and fat soluble vitamins, survived for $1^1/_2$—$3^1/_2$ years, investigated the serum phosphatase in one dog and did not observe any increase.

Comment: This work, whose main task is not phosphatase studies, proves the superiority of the internal closed method, when long survival is wanted. This case is a single, but important one.

Different Techniques in Making Biliary Fistulas.

Bile fistulas are easy to make but difficult to maintain. This is confirmed by various authours who have worked with biliary fistulas for other purposes (Rous and McMaster (1923) and Snape, Wirts and Cantarow (1947) with further references). Short and wide fistulas involve ascending infection in a few days. With fistulas from the gall bladder, one cannot always be sure that the passage through the cystic duct is free, and if rubber tubes are passed through the cystic duct to the common bile duct (Drill et al.) their opening may pass downwards instead of upwards.

The method involving the least disturbance to the function of the biliary excretory apparatus unquestionably is that of Thomas 1941, comp. Snape, Wirts and Cantarow (1947) in which the bile duct can be probed directly through a larger fistula opening into the duodenum; while the usage of "internal fistulas" that means cholecysto-nephrostomia, renders a long survival possible. As the author has wanted to collect the bile continuously, however, these methods are inconvenient here. The author has chosen, therefore, to insert the fistula tube direct into the common bile duct using a modified Rous-McMaster technique.

Material, Surgical and Analytical Techniques.

15 grown dogs were used. They were fed rye bread with margarine, milk and glucose solution, except for a starvation period of 18 hours previous to the operations.

The operations were performed either in ether or in narconumal anesthesia under aseptic conditions, through mid-line sections. The common bile duct was doubly ligated immediately before the entrance into the duodenum. To avoid the last hepatic duct on the right side it was necessary to insert the tube so far down as possible in the common duct, although it was not always widest here. Small tubes of stainless steel, brass, lac or glass of different length were tried, but the experiments proved, that short, wide glass tubes with a little constriction (mouth-pieces for pipettes) were the best means to avoid tissue irritation with obstruction of the fistula through mucous or bile pigment or catching of the point in the wall of the duct.

A rubber tube of about 30—35 cm length and of suitable diameter (about 4 mm) had been connected to the glass tube in advance and was now placed in a large curve inside the peritoneal cavity, not interrupted by glass tubes (as used by ROUS-MCMASTER). The operation field was covered by omentum, which in a few hours would circumvaginate also the rubber tube, thus arresting possible infections. The tube was drawn out by means of a péan through a minute incision to the right or left of the middle line. The end of the tube was connected to a small glass tube with a collar to which was tied a rubber bag for the collection of bile.

The wound was drenched with mastix and dressed with aseptical gauze kept in place by means of plaster and left untouched as long as possible. A cover of canvas, tied on the back of the dog and with holes for the forelegs, was found to be essential in order to avoid that the dog lacerated bag and tube.

The serum phosphatase was estimated a few times before and each day after the operation, by means of the modified BUCH and BUCH, method (1939) as previously described (DALGAARD 1948), but only 100 mm^3 of serum and 15 minutes of hydrolysis was used. Icterus index (Meulengracht) was estimated quite often. The bile was quantitatively collected at 24 hours' intervals and the phosphatase content estimated as in serum but after dilution with saline to $^1/_{10}$. The colour of the bile was not considered as it was diluted 750 times before the reading and the blank contained the same amount. The bile was often analysed even undiluted with saline and good agreement found.

In the tables are stated the amount of bile (g, in cc and the total amount of phosphatase in BUCH and BUCH units. The calculation is as follows:

$$\text{Total phosphatase} = \frac{g \cdot e \cdot k \cdot 10}{50} = g \cdot e \cdot 5.6,$$

where e is the extinction on the scale of the Pulfrich photometer and k is a constant, estimated by means of known phenol standards, here = 28.

Table

In the following tables "Serum phosphatase" means the phosphatase activity, means the total phosphatase excreted in the bile
A 0 means that nothing is excreted, while a ? means that the amount was not means that it is a minimum value. The serum

Days after operation:	1	2	3	4	5	6	7	8	9
Serum phosphatase ($7^1/_2$, 5).	4	7	$5^1/_2$	2	7	3		$15^1/_2$	17
Amount of bile.....	80	50	80	100	150	220	160	?	0
Phosphatase in bile.....	16	37	$8^1/_2$	95	294	642		?	0
Icterus index.....		10		10	10	10			10

H_{46}, police dog, 30 kg. The serum phosphatase was normal as long as the fistula was properly functioning. On the 26th day the dog pulled the fistula out. See the text.

Criteria for the Sufficiency of Bile Fistulas.

Bile fistulas, as previously mentioned, easily pass into the opposite: bile stop, partial or complete. Therefore it is necessary to put forward strict and rigorous demands to the experiments.

The first demand must be a free flow of an abundant amount of bile.

BERMAN et al. (1942) in mean obtained 126 cc daily (55 estimations in 15 different dogs). But the amount, of course, will vary with the size of the dog. BOYD et al. (1945) stated 7.3—13.2 cc/kg and day as a norm, while STADELMANN (1891) in his studies from the literature met values between 3 and 36 cc/kg and day, and in his own material obtained 9—22 cc/kg and day. MCMASTER, BROUN and ROUS (1923) finally, got 1—14 cc/kg and day. The present author gets figures which correspond to the last mentioned ones, see below.

In practice the most important point is that suspicion is raised when a dog suddenly diminishes its bile output.

The first day or two the amount of bile is scanty as a consequence of the operation trauma, eventually even an effect of the morphine (1 cg/10 kg) given preoperatively and especially because the animals eat nothing or only little during this time. Comp. the similar findings of MCMASTER, BROUN and ROUS. This is the reason that the phosphatase excreted in the bile during the very first days cannot serve as a base for calculations of phosphatase retention in ordinary obstruction-dogs. (See further on p. 2 and p. 11.)

The second demand is a normal plasma colour, that means an icterus index below ca. 15. An increase in the icterus index in all

1.

expressed in Buch and Buch units/50 cc of serum, while "Phosphatase in bile" during 24 hours (bile amount × phosphatase activity).
measured for some reason (mostly because of bag-leakage). A > before a figure phosphatase figures in ( ) are the preoperative ones.

10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
25	23	20	20	14½	15	12	11	12	14½	11	12	11	11		15	14½	19½
0	?	?	150	30	0	65	80	200	0	0	?	> 60	380	380	320	see text.	
0	?	?	104	0	0	80	102	21	0	0	?	> 64	144	?	34		
8		10	5	6	8	6				9	9		8		6	9	

probability means that mechanical obstruction is at hand and should be assigned much importance. In long standing fistula dogs an increased icterus index may indicate beginning cirrhosis of the liver.

The third demand must be that the animal goes on well, is lively and moves around normally. The bile must be of normal colour and smell and not be visibly infected or purulent.

Results.

Of the 15 dogs used, 2 died on the first day, from shock and abdominal hemorrhage, respectively, and one died from peritonitis on the third day. The remaining dogs recovered well from the operations. During the following weeks some loss of weight occurred. Most animals have been killed when obstruction to the bile flow occurred, in order to obtain exact autopsy diagnoses. H_{28} and H_{30}, however, were left alive for 75 and 10 days, respectively, after the stopping.

Most animals sooner or later developed increase in the serum phosphatase. This preliminary "result", however, must be thoroughly analysed, or else the whole investigation will be in vain. According to the demands stated above for considering a fistula as sufficient, it is necessary to study the phosphatase level in each dog *before* and *after* the occurrence of complications (obstruction), as only the former condition represents true fistulas.

Case reports. A few typical experimental results shall be presented.

H_{46}, a large police dog weighing 30 kg recovered easily from the operation and soon excreted appreciable amounts of bile (100—200 cc/day). The serum phosphatase was quite normal. On the 9th day an occlusion of the fistula occurred, which was successfully overcome 3

days later by means of rinsing with saline. Concurrently with this stopping a slight transient increase in the serum phosphatase could be noticed. After this the fistula functioned well until the 26th day, when the dog pulled the tube out and was killed next day. At the autopsy bile was found in the peritoneal cavity but the gall bladder and common bile duct were not dilated.

Conclusion: In this dog both the amount of bile, icterus index and general condition as well as the autopsy proved that the fistula was allright. The serum phosphatase was normal, apart from the transient slight increase in conjunction with the stopping.

In the dogs in tables 2 a and b, H_{54}, boxer, 25 kg and H_{39}, spitzdog, 13 kg, the fistulas were functioning well for one week. In the former the amount of bile was rather copious, in the latter less, even when the difference in size was considered. The serum phosphatase in both was within normal limits. After a week H_{54} pulled his fistula tube out and was killed. The autopsy displayed an uncomplicated fistula case. H_{39} became suddenly ill on the 8th day. The autopsy showed that the fistula tube, partly filled with bile pigment, had perforated the common bile duct with cholascos as a consequence.

Table 2 a.

Days after operation:	1	2	3	4	5	6	7
Serum phosphatase (4, 6½)	12	1	15		11½	10½	7½
Amount of bile.....	80	110	?	250	240	?	?
Phosphatase in bile	138	53	?	720	425	?	?
Icterus index.....	5		10		12	10	

Table 2 b.

Days after operation:	1	2	3	4	5	6	7	8	9
Serum phosphatase (6½, 5½)	18	13	13		7	13	18	86	128
Amount of bile.....	0	120	30	> 50	70	45	60	90	0
Phosphatase in bile.....	0	57	8	> 11	21	127	213	105	0
Icterus index.....	15						15	20	25

In two dogs, H_{54}, 25 kg. and H_{39}, 13 kg, the fistulas functioned well for 7 days, The serum phosphatase was normal, although a little above the preoperative level. On the 7th day H_{54} pulled the fistula out and was killed, while H_{39} became suddenly ill on the 8th day, because the fistula tube had perforated the common duct with cholascos as a consequence.

In H_{26}, police dog, 25 kg, tables 3 a and b, the amount of bile and the amount of phosphatase excreted was appreciable. Sometimes the dog succeeded in biting the rubber bag, which is the reason for the missing figures in the table, but the excretion was hardly diminished during such days. A transient stop was also in this dog followed by a

transient increase in phosphatase but otherwise the phosphatase was at a normal level. On the 25th day a stop occurred, which could not be overcome and the phosphatase increased considerably and continuously. On the 37th day, after 12 days of obstruction, while the phosphatase was at a very high level, a reoperation was performed, and a new, short fistula was inserted into the expanded gall bladder. This new fistula functioned well during the following days, with increasing output of phosphatase through the bile and with decreasing phosphatase in the serum. At last an ascending infection developed and the dog was killed 9 days after the second operation. The autopsy proved that the new fistula had been all right, but that the first fistula was completely stopped and imbedded in adherences of omentum. Concerning the phosphatase excreted through the bile, see below.

In the large table 4 are collected the results from 5 dogs displaying complications, total (H_{36}) or partial obstruction ($H_{51, 28, 30, 41}$). In the table the small and varying amounts of bile can be seen and the increases in icterus index. The most important autopsy findings are stated also. In all cases increases in the serum phosphatase are obvious, most distinctly in H_{36} which in reality is not a fistula dog but a bile obstruction dog.

It is unnecessary to deal with the remaining dogs in detail, as they show results which vary between the examples given already. But it shall be stressed, comprehensively, that when this comparison between uncomplicated fistula dogs and complicated ones is completed, it is found that in every case when the fistula is all right, the serum phosphatase is normal, although sometimes near the upper limit of the normal interval. This condition is seen quite convincingly whether the fistula has been functioning for a short or long time (here maximal 25 days).

Phosphatase in bile.

The amount of bile and especially its phosphatase content vary very much. The volume may rise to 380 cc/day or 13 cc/kg and day and the mean of uncomplicated cases is 137 cc/day or 6.5 cc/kg and day. Considering the amount of bile it should be remembered that the gall bladder is not removed and a certain degree of concentration may have occurred.

The phosphatase activity varies appreciably, between 0 and 185 units/50 cc, with 3 single estimations exceeding this appreciably, up to 1 700 units. The variations in the total phosphatase output, of course, are even larger, between 0 and 2 850 units/day

Table

Days after operation:	1	2	3	4	5	6	7	8	9	10
Serum phosphatase (16) .	19	17½	15	9½	14	5	3		10	11
Amount of bile.	30	15	60	240	110	150	> 120	?	?	> 100
Phosphatase in bile.....	80	37	2 000	2 850	490	382	> 400	?	?	> 800
Icterus index.				10	10		20			20

Table 3 b.

Days after 2nd operation:	1	2	3	4	5	6	7	8	9
Serum phosphatase (91, 82)	53	106	85	85		61	75		45
Amount of bile.....	60	110	200	> 100	240	> 120	65	60	15
Phosphatase in bile.....	215	350	382	> 120	488	> 400	140	900	157
Icterus index.....	30	20	20	20		10	10		15

H_{26}, police dog, 25 kg. Serum phosphatase practically normal as long as the fistula was functioning, but increased considerably when obstruction occurred. After 12 days of obstruction another fistula was made and the serum phosphatase decreased, while the phosphatase output through the bile increased. See the text, esp. concerning the bile phosphatase.

or in maximum 114 units/kg and day. The mean total phosphatase output is ca. 300 units/day.

In spite of the very large variations certain observations can be made: The very first days after the operation the output is limited and only exceptionally exceeds 100 units/day. This is, without doubt, a consequence of the scanty amount of bile during this time (see above). Moreover it is distinctly seen that only dogs with sufficient fistulas, according to the demands given above, display an appreciable output of phosphatase, while, on the contrary, just the dogs with limited phosphatase output through the fistulas, display the largest increase in serum phosphatase.

It is of some interest to follow H_{26} after the second operation, when the serum phosphatase was high and the phosphatase output increased each day. It is reasonable to assume that on the 4th day the output had reached a niveau, exceeding 400 units/day, where the new formation of phosphatase was compensated and the serum phosphatase began to decrease. It is tempting in this dog to try a calculation: If we consider the maximal phosphatase output, about 2 500 units, and suppose this amount not to be excreted but instead to be retained in the animal and distributed in the serum, maximal ½ liter, this would correspond to an in-

3 a.

11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	31	32	37
11½	12	8½	14	18½	61	7	16		15		18	15	15	13	14	60	107	91	82
50	100	?	0	0	0	?	?	?	80	120	>100	0	0	0	0	0	0	0	0
111	85	?	0	0	0	?	?	?	34		>32	0	0	0	0	0	0	0	0
	10								15		5					50			40

crease in serum phosphatase of 250 units/day. This easily explains the increase in serum phosphatase which actually occurs during obstructive jaundice.

More interest than this isolated case it should have to carry through the same calculation on the whole material, as expressed in the mean value.

If thus the mean daily output of phosphatase through the bile, 300 units, should be retained in an animal of about 20 kg and distributed in its serum only, this would correspond to an increase in phosphatase about 25 units/day. As is seen from another paper of the author, it is just a phosphatase increase of this magnitude, or less, that is met with in simple obstruction of the common bile duct.

Addendum: A Case of Duodenal Fistula.

The biliary fistulas shall here be supplemented with a case of duodenal fistula which operation must be supposed to influence the function of the biliary excretory system less directly than operations on the gall bladder or the common bile duct themselves.

Technique: In an 8 kg dog in ether anesthesia the duodenum was divided just inferior to the pancreas and a coarse tube (1 cm diameter) was inserted into the upper end while a fine tube was inserted into the lower end of the bowel. Both tubes were led out on the surface of the abdomen. Through the wide fistula, which was left open, the bile flowed, as well as, of course, the ventricular, duodenal and pancreatic juices and the food. Through the small rubber fistula the dog could be fed with glucose, saline and amino acids. Sulphatiazol was given also. In all, 300 cc of fluids was injected daily. The secretion from the duodenal fistula was not collected.

The results of the phosphatase analyses, performed daily, are seen from table 5. The icterus index (3rd, 4th and 5th day), the thymol test (2nd and 6th day) and the plasma bicarbonate

Table 4.

Dog no.	Days after operation:	1	2	3	4	5	6	7	8	9	10	11	12	13	Remarks and autopsy findings.
H_{31} 30 kg	Serum-phosphatase (3½, 5)	12	17	23	19½	18	32		36	32	36	30		22	Partial mechanical obstruction, caused by sedimentation of bile pigment in the fistula-tube.
	Amount of bile	20	20	30	80	80	120	?	150	100	30	60	40	65	
	Phosphatase in bile	4	83	272	144	81	122	?	128	48	40	139	72	28	
	Icterus index				10	15	20		25	20				14	
H_{30} 11 kg	Serum phosphatase (3, 2½)	4	32	107	107		85	64	140		55	75		61	Partial mechanical obstruction, caused by catching of the inner end of the fistula tube in the wall of the common duct. This and the gallbladder dilated.
	Amount of bile	0	35	10	40	50	80	60	50	55	60	80	80	0	
	Phosphatase in bile	0	280	66	82	90	127	108	370	450	121	390	161	0	
	Icterus index		15		25		25	45	50			30			
H_{41} 9 kg	Serum phosphatase (7, 7)	18½	70	65	85	96		200							The dog became suddenly ill on the 8th day and was killed. The autopsy showed dilation and perforation of the common bile duct with bile peritonitis.
	Amount of bile	0	20	40	40	0	0	10							
	Phosphatase in bile	0	93	161	93	0	0	22							
	Icterus index			20	25	20		25							
H_{28} 15 kg	Serum phosphatase (10)	11	8½	15½	37		59	57	107	160	67			> 160	This dog survived for two months further with intense jaundice, complete stop to the bile flow and serum phosphatase steadily above 160. Autopsy: total stop.
	Amount of bile	40	65	?	50	10	5	0	0	?	?	5	0	0	
	Phosphatase in bile	16	109	?	49	3	2	0	0	?	?	2	0	0	
	Icterus index		12		15			40	60						
H_{38} 11 kg	Serum phosphatase (3, 3)	19	56	59	59	61	64	64	> 160	> 160					Intense jaundice, Autopsy: Complete stop, dilated common duct and gall bladder.
	Amount of bile	0	0	0	0	0	0	0	0	0					

In 5 dogs the biliary fistulas were partially or completely obstructed. Tho serum phosphatase increased, especially when tho obstruction was completo.

Table 5.

Days after operation:	1	2	3	4	5	6
Serum phosphatase (7)	12	19	11	14	11	17

H_{23}, 8 kg. After the performance of a complete duodenal fistula the dog emaciated. The serum phosphatase was slightly increased, but still within (or near) normal limits.

(4th and 6th day) were all normal. The autopsy displayed only emaciation and localized infection around the inferior fistula.

Comment: This case of duodenal fistula, which indirectly is even a biliary fistula, displays a slight increase in serum phosphatase, which, however, hardly exceeds the normal level, and perhaps partly may be explained through the drying out caused by the fistula operation.

One more operation of this kind failed, as the dog pulled the fistulas out and developed a putrid infection.

Discussion.

The experiments have shown that when the bile is withdrawn through a common bile duct fistula, which functions well, or indirectly through a duodenal fistula, no increase in serum phosphatase, or at least only a very slight increase occurs. An increase of the magnitude which is known from ordinary biliary obstruction dogs occurs only in cases where the fistula is quite stopped and thus in reality is not a fistula any more.

This result is in itself less surprising than those of certain previous authors obtaining the opposite conclusion. As already stated in the comments to these, several cases obviously have not been properly functioning fistulas in the meaning described above. Maybe, complications even have been at hand in some other cases, where the statements are less complete.

A strong support to the findings of the author are the findings of Jalling, Laursen and Volqvartz (1945) in rabbits. It is unlikely that great discrepancies between rabbit and dog should exist in this direction.

Drill, Annegers and Ivy (1943) said that operations on the biliary tract (cholecystectomy) was followed by a disturbed function of the liver. This conclusion was based upon their finding of increased phosphatase and bromsulphonphthalein retention.

The author's findings concerning the phosphatase is described above. Bromsulphonphthalein retention has not been studied here but the thymol test was sometimes performed, always with normal results. The findings of the author thus give no support to the statement of DRILL et al.

As a consequence of all the information available but with particular stress on his own results the author feels entitled to make the following conclusions which conform well to the results in a previous paper, where dogs without intestines but with ligated common bile duct displayed the same increase in serum phosphatase as ordinary biliary obstruction dogs.

Conclusions.

1) In dogs with uncomplicated biliary fistula without biliary stasis, the serum phosphatase does not increase, or increases very slightly.

2) The increased serum phosphatase in dogs with obstruction of the common bile duct is not caused by the absence of bile in the intestine.

3) In the present material the dogs with properly functioning biliary fistulas excreted so much phosphatase through the bile that its complete retention might be sufficient to explain the phosphatase increase during biliary obstruction.

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Serum Phosphatase after Hepatectomy in Dogs.

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In order to evaluate the relation of the liver to the increase in serum phosphatase following obstruction of the common bile duct, it is necessary to study the serum phosphatase after hepatectomy. In fact, this seems to be the only way of determining if the phosphatase increase is of hepatic or extrahepatic origin.

Previous Investigations.

Partial removal or destruction of the liver for investigation of the phosphatase has previously been done by various methods. In every case a phosphatase increase of varying degree was observed.

Partial surgical removal was done by FREEMAN, CHEN and IVY (1938) and by OPPENHEIMER and FLOCK (1947, rats). The former also performed destruction by means of inoculation of leptospira icterohaemorrhagica.

Clinically besides the considerable phosphatase increase in cases of obstructive jaundice, a transient and moderate increase is often seen in infectious hepatites, and even in cases of hepatic cancer or cirrhosis.

Chemical agents with poisonous effect on the liver have been widely applied: Carbon tetrachloride (HARTMAN and SCHELLING 1934, BODANSKY 1937, FREEMAN, CHEN and IVY 1938, SEHRA, CHOPRA and MUKERJI 1941, DRILL, ANNEGERS and IVY

1944, DRILL and IVY 1944—45); Toluylendiamine (ARMSTRONG and KING 1935, BODANSKY 1937—38); Hydrazine (BODANSKY 1937); Chloroform (ARMSTRONG and KING 1935, BODANSKY 1937, BALCELLS-GORINA and SOLS 1946); Arsenic (SCHIFFMANN and WINKELMAN 1939); Neoarsphenamine (BODANSKY 1937) and Phosphorus (ARMSTRONG and KING 1935, BODANSKY 1937). Intravenous injection of gum acasia (BODANSKY, 1939) has also been used.

Destruction of the liver by means of X-rays of high voltage in dogs with Eck's fistula was also followed by phosphatase increase, according to HARTMAN and SCHELLING (1934).

Our main problem, however, is unsolved through all the above results: Is the increased phosphatase caused by the lack of phosphatase-excreting liver tissue — or in the contrary released from the damaged liver remnants? Only complete hepatectomies can give the answer.

Complete removal of the liver is a technically difficult procedure which has been attempted only twice for phosphatase studies, and both reports are extremely short.

ARMSTRONG and BANTING (1935) removed various organs in dogs expecting a decrease in serum phosphatase to occur if the organ of the normal formation of the enzyme was excluded. However, after removal of the liver or after evisceration including hepatectomy, a considerable increase was observed and they concluded that "bone is the sole source of serum phosphatase".

MADDOCK, SCHMIDT and THANNHAUSER (1942) "found that hepatectomized animals (dogs) showed a marked rise in phosphatase in the postoperative period. Abdominal evisceration without hepatectomy produced no rise and abdominal evisceration with hepatectomy gave a slight rise." No details about the technique, the completeness of the operations or the length of survival were stated, nor were any phosphatase figures published. Intravenous injection of a highly purified phosphatase solution which raised the serum phosphatase to 400 Bodansky units, was in normal dogs followed by a sharp fall, but by a progressive rise to about 1000 units in the hepatectomized animals. "These results were interpreted as indicating that the liver acted as a regulator of phosphatase in the serum."

Thus the work done till now implies that the destruction or removal of the

liver is associated with increased serum phosphatase, but both reports on total hepatectomy are very brief and give no details. No investigation has so far been performed of the phosphatase after "functional hepatectomy" (see below).

Different Techniques for Hepatectomy.

The anatomical relations of the liver, especially its double blood supply, involves two major difficulties by its surgical removal: The blood from the portal vein and from the inferior cava vein must be returned to the heart.

I. Functional hepatectomy. The difficulty of the portal system is overcome by means of an Eck's fistula, *i. e.* an anastomosis between the cava and the portal veins with ligature of the latter superior to the anastomosis (Eck 1877). After this the hepatoduodenal ligament can be tied, thus depriving the liver completely even of its arterial blood supply. The only connection with the vascular system is now the hepatic veins, joining the inferior cava vein, but only a minor diffusion will pass through this route and an incision into the liver parenchyma will not even bleed! Unfortunately, however, autolysis of the liver releases poisonous products which cause the death of the animal within a few hours.

Such functional hepatectomy, with the liver left in place, has here been performed in three dogs.

II. Real hepatectomy. Complete surgical removal of the liver can be accomplished in two essentially different ways.

The three-stage method of Mann (1921) utilized a collateral circulation to maintain the venous return. First the cava vein was ligated just above an anastomosis made to the portal vein ("reverse Eck's fistula"). The increased venous pressure then forced collaterals to develop, primarily through the azygos and internal mammary veins. A few weeks later the portal vein was ligated, and three months after the first operation the liver could easily be completely removed.

This method proved of inestimable value for the study of liver physiology but its long duration is a drawback. Modifications which can be performed in two stages (Markowitz and Soskin 1927) and in rabbits (Drury 1929, Himsworth 1938) have been described.

In the second method, originally devised by Firor and Stinson (1929) the blood from the cava vein was led through a wide T-tube of glass, replacing the part of the vein imbedded in the liver. The portal vein was connected to the side tube of the T, which thus constituted a veritable Eck's fistula. This method is possible only because hepatectomy involves delayed coagulation of the blood. (For technical details, see the original paper.) — The author has tried this method without much success.

The same principle with a glass tube replacing the cava vein is utilized in the method devised by MARKOWITZ, YATER and BURROW (1933). As blood stasis is nearly completely avoided and the hepatectomy can be performed in one stage, this method has been chosen in the present investigation.

The large operation involves three sub-stages: 1) Through a minute incision in the cava vein, just beneath the liver, a wide glass tube, without side tube, is inserted and the vein at once tied to it. The tube reaches in the vein just above the diaphragm. 2) An anastomosis of 2 cm length is made between the portal and the cava veins, just below the tube. By the ingenious method of FISCHLER and SCHRÖDER (1909) and FISHBACK (1927) obstruction or opening of any of the two vessels can be completely avoided. 3) After clamping and cutting the hepato-duodenal ligament, cutting the ligaments to the diaphragm and tying the cava vein with the hepatic veins around the glass tube immediately below the diaphragm, the liver can now be totally removed. The liver bed is filled out with stuff to offer resistance to the diaphragm and the wound is suturated.

It has been a matter of much trouble and demanded many trials for the author to learn this method. The operations lasted about two hours. The cava vein was obstructed less than two minutes. This operation was successfully performed in two dogs.

III. Evisceration. This operation is technically more simple although physiologically more complicated. A glass tube is inserted into the cava vein as in stage 1) above. The rectum is then divided and invaginated and the large intestine, small intestine, ventricle, pancreas, spleen and omentum are removed en bloc, the large arteries being ligated near their origin from the aorta. Finally the liver is removed as in stage 3) above. This operation was performed in one dog and lasted one hour.

Previous to all operations the dogs were starved for 24 hours, the importance of which is stressed by MANN and others. The operations were aseptically performed in ether anesthesia through large S-shaped incisions. To assure quick awakening no morphin but only 0.5 mg of atropin was given preoperatively to diminish the saliva. As a precautionary measure to prevent blood clotting, 10 mg of heparin was injected into the cava vein in the methods involving its opening.

General Effects of the Hepatectomies. Glucose Supply.

The general and numerous special effects of hepatectomy are described in several works especially by MANN and collaborators. Hence the author wants only to stress the most important reactions and his measures of controlling them.

The dogs wake up immediately after the operations and after a few minutes they usually get up and walk around. They may now act apparently normally for a few hours. They move, drink water but take no food (nor should they be allowed to do so because of the danger

of protein shock). The dogs obey when called and may even wag their tails.

After 2—5 hours the dogs rather suddenly display profound weakening, are unable to stand upright but lie down quietly, and soon after obey no calling. Suddenly muscular twitchings set in, at first of a single leg or of the face but soon after generalized clonical and at last tonical cramps occur with extensor-tonus and opistotonus, resulting in death in a few minutes.

The symptoms are due to hypoglycaemia and are very similar to insulin shocks. All symptoms, even the cramps, can be overcome by intravenous injection of glucose which have an immediate and dramatic effect. The moribund dogs rise and walk around as before.

To prevent cramps and death in hypoglycaemia the dogs were given glucose intravenously, in 20 per cent solution at hourly intervals, and to a small extent even orally and subcutaneously (glucose-saline solution). In acute situations dogs in cramps have sometimes been saved only through intracardial injections.

Blood sugar was determined 1—2 times per hour and decreased from 150—125 mg% shortly after glucose infusions to sometimes less than 30 mg% about 1 hour after the infusions. The blood sugar, however, afforded only limited guidance for the glucose dosage, as the hypoglycaemic symptoms occurred at steadily increasing blood sugar levels, and in acute situations even the quick Creselius-Seifert method for blood sugar determination required too much time. The treatment was started with $^1/_4$ g of glucose per kg and hour, according to MANN, but the dosage had sometimes to be augmented according to the clinical symptoms. After some hours even glucose therapy proved without any effect, and the dogs died, usually during attacks of cramps. To save space blood sugar figures and glucose dosage are indicated for only one dog, H_{77} (fig. 3).

The icterus index (Meulengracht) always displayed a marked increase after the operations, in one case (H_{77}) from 6—50 within 10 hours.

The autopsies proved that the operations had been successful; without thrombosis either in the cava vein or the glass tube or in the portal system. No noteworthy bleeding in the abdominal cavity had occurred and the hepatectomies were complete, as less than 1 g of liver tissue was left.

The dogs with functional hepatectomy displayed the same symptoms as the real hepatectomized dogs and their livers were found pale and anaemic at the autopsies.

Results.

The phosphatase was estimated as described in previous publications. Blood was withdrawn the day before the operation and after 24 hours of fasting immediately before the operation and

at hourly intervals after the operation. The results are seen in the figures 1—4.

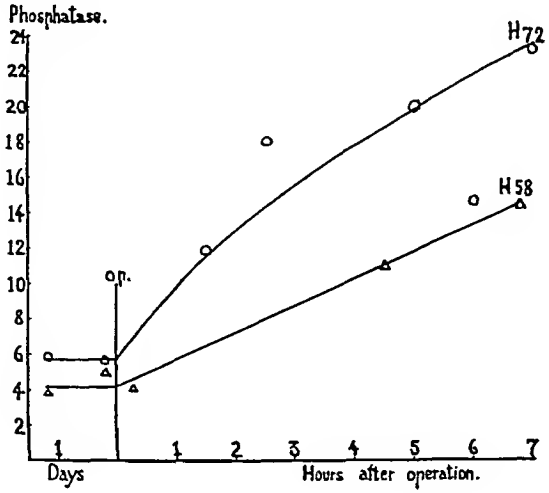

Fig. 1.

H_{58} and H_{72}, two cases of functional hepatectomy in each of which a distinct increase in serum phosphatase is seen.

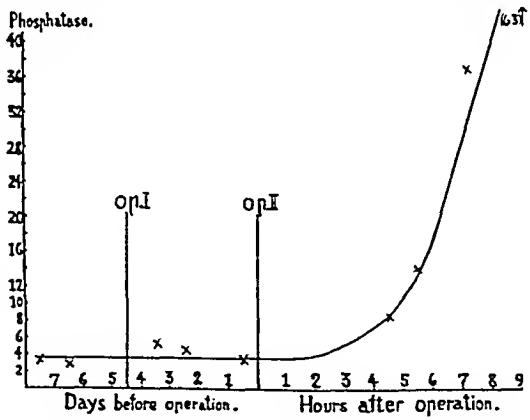

Fig. 2.

H_{59}, functional hepatectomy performed in two stages. At first only an Eck's fistula is made (op. I) which does not seem to influence the phosphatase. But following ligation of the porta hepatis, five days afterwards (op. II), a distinct rise in serum phosphatase occurs. (Note that the scale is only half size.)

Fig. 3 shows two cases of real, complete hepatectomy. In H_{75} the phosphatase increases enormously within only 4 hours! In

H_{77} the increase is less abrupt but steady and considerable, reaching four times the normal level within 10 hours. For this dog the blood sugar figures are indicated (in italics). The columns

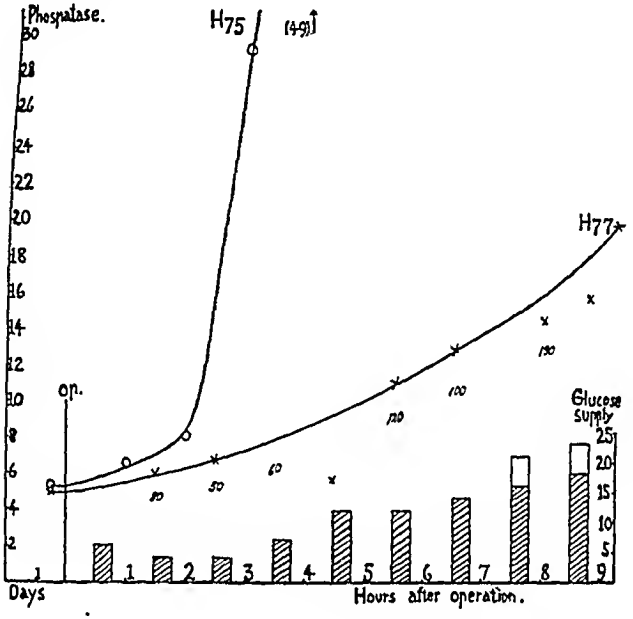

Fig. 3.

indicate the glucose supply per hour, intravenously (grey columns) and subcutaneously (white columns). Note that glucose is supplied immediately after each blood sampling, used for both phosphatase and blood sugar determinations, while the cannula is still in the vein. The blood sugar is therefore not known until about 10 minutes after the glucose supply and can therefore afford guidance only to succeeding glucose dosages.

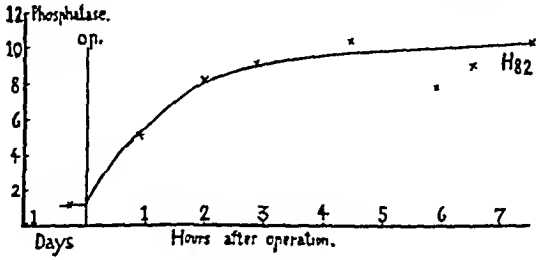

Fig. 4.

H_{82}, a case of complete evisceration, including hepatectomy but without nephrectomy. A distinct rise in serum phosphatase is seen, although it seems to be a little less than in the former cases.

H_{92}, control experiment. Hourly injections of 20 per cent glucose solution intravenously in a normal dog is without any effect on the serum phosphatase, which keeps quite constant. The dog was starved for 40 hours previous to the experiment

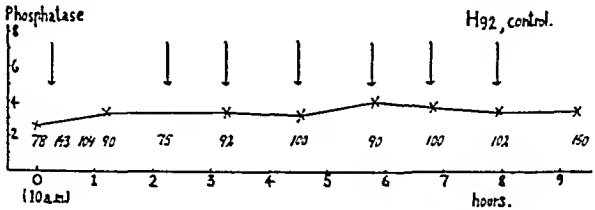

Fig. 5.

to assure a minimal phosphatase level. Each arrow means injection of 3 g of glucose (weight of dog 12 kg). Figures in italics mean blood sugar levels.

Discussion.

The results of the functional hepatectomies, the real, complete hepatectomies and the evisceration thus all conform to the results of previous investigators, as referred to in the introduction: Hepatectomy is followed by an increase in the serum phosphatase.

The increase widely surpasses the normal limits of phosphatase in dogs (see further in a coming paper) and is significant. But the experiments involve very unphysiologic conditions, is it possible to draw conclusions from these? The same objection was originally advanced against many results obtained by hepatectomies, which later proved to be correct. The fact that the phosphatase reaction is positive (increase) also points to its correctness and specificity, as a negative reaction (no increase) would more likely be an unspecific sign of a damaged organism.

The influence of anesthesia, operation trauma or shock was ruled out in several control experiments (not stated here) with other extensive operations (ventricular or intestinal resections a. o.) which displayed no phosphatase increase. In the control experiment fig. 5 it was also proved that hourly injections of glucose did not influence the phosphatase level.

The results, of course, do not exclude the possibility that the intact liver during certain circumstances, as regeneration, may yield some phosphatase to the blood. However, the finding of increased phosphatase in the remnants of partially removed

(rat-)livers (OPPENHEIMER and FLOCH 1947) need not mean that the enzyme is formed here. It may just as likely be accumulated because the liver remnants are overstrained in the effort to excrete all the phosphatase ordinarily excreted by the whole liver.

It is astonishing that the speed and intensity of the phosphatase increase after hepatectomy widely exceeds the increase occurring when the common bile duct is ligated. If cholecystectomy is performed at the same time, however, an essentially higher increase follows, although still less than after hepatectomy (see a coming paper).

As bile is very rich in phosphatase it is reasonable to assume that the higher speed with which serum phosphatase increases after bile duct ligation with cholecystectomy depends upon the absence of the storing capacity of the gall bladder, and after hepatectomy even of the hepatic ducts, bile capillaries and liver cells.

These considerations strongly point to the conclusion that the increased serum phosphatase after ligation of the common bile duct is of the same extrahepatic (osseous) origin as the increased phosphatase after hepatectomy.

Summary.

In numerous experiments of previous authors partial removal or destruction of the liver by means of surgical, infectious, chemical or physical methods was followed by increased alkaline serum phosphatase. But the rôle of the remaining liver tissue was always difficult to judge. Phosphatase increase following hepatectomy has been reported twice, but both reports, although important, are very brief and give no details.

The author excluded the liver in dogs: 1) through ligation of the hepatic artery in dogs with Eck's fistula (functional hepatectomy), 3 dogs; 2) through complete hepatectomy in one stage, 2 dogs; and 3) through complete evisceration in one stage, 1 dog. The dogs were kept alive by intravenous glucose supply, and the phosphatase estimated at hourly intervals.

Although the dogs survived for only 5—10 hours, a *distinct rise in serum phosphatase was observed in all cases* (curves 1—4). In control experiments it was proved that neither the anesthesia, other large operations nor glucose supply (curve 5) was associated with phosphatase increase.

The speed and intensity of the phosphatase increase is ex-

plained by the absence of the storing capacity of the gall bladder, ducts and liver parenchyma.

Although it is not excluded that the intact liver sometimes may yield phosphatase to the blood, the investigation indicates that the rôle of the liver in phosphatase regulation is primarily as a regulator of phosphatase excretion. The investigation proves that a marked and quick phosphatase increase can be of purely extrahepatic origin and imply that the increase following obstruction of the common bile duct is of the same extrahepatic (osseous) origin as the one observed here.

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Serum Phosphatase after Nephrectomy and Bile Obstruction in Dogs.

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In order to decide which organ is the source of the increased serum phosphatase during obstructive jaundice, the author has performed complete removal of the small intestine with ligation of the common bile duct and complete hepatectomy with or without abdominal evisceration (excluding nephrectomy) in dogs (1949 b and c). In all cases a distinct *rise* in serum phosphatase occurred which consequently must have originated from another source.

As renal cortex is extremely rich in alkaline phosphatase, it is necessary also to consider the possible significance of the kidneys. The phosphatase is here confined to the proximal convoluted tubules and in all probability engaged with glucose reabsorption, but an additional function could not be excluded.

TAKATA (1932) and HOFFMEYER, JALLING and SCHÖNHEYDER (1946) observed a significant *decrease* in phosphatase activity of renal cortex during obstructive jaundice *in rabbits*, but removal of the kidneys did not prevent phosphatase increase. GAD (1946) demonstrated an insignificant *increase* in jaundiced *dogs*.

Removal of the kidneys and ligation of the common bile duct *in cats* was not followed by phosphatase increase (DALGAARD, 1948 a) but this might be explained through the slight increase ordinarily observed in cats, possibly in connection with the inhibitory effect of urea on phosphatase activity.

Simple nephrectomy *in dogs* was without influence on the serum phosphatase (ARMSTRONG and BANTING, 1935), whereas simultaneous bile obstruction caused a phosphatase increase.

Experimental.

In the present study double nephrectomy with ligation of the common bile duct was performed in two dogs and nephrectomy without touching the duct in two other dogs (controls). Serum phosphatase, blood urea and icterus index (Meulengracht) were estimated before and each day after the operation. For phosphatase method, see previous papers (DALGAARD 1948 a and b).

Dog.		Before	1. day	2. day	3. day	4. day
H_{99}	Phosphatase Blood urea	5.0	14 (93)	55 (200)	122 (215)	168 (440)
H_{98}	Phosphatase Blood urea	3.0	5.0 (102)	4.7 (195)	4.2 (270)	4.0 (>500)

Nephrectomy with ligation of the common bile duct (H_{99}) was followed by a considerable increase in serum phosphatase and blood urea. Icterus index (not stated) increased steadily to 110.

Nephrectomy without ligation (H_{98}) proved without influence on the serum phosphatase. Icterus index remained normal, but blood urea increased, of course, as before.

In two other dogs, not stated here, quite similar results were obtained.

In vitro experiments with dog serum, as previously with cat serum, displayed that urea inhibited phosphatase activity, depending upon the concentration of urea and the duration of its action on the serum. Such inhibition, however, is not visible in the above experiments, as it is negligible compared to the enormous increase following ligation of the common bile duct. The inconspicuous decrease in H_{98} is without any significance.

Summary.

Through nephrectomy with ligation of the common bile duct it is proved that the kidneys are without influence on the increased serum phosphatase during obstructive jaundice in dogs.

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Effect on Blood Pressure of Enzymatic Casein Digest.

By

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Received 18 June 1948.

During the last ten years the parenteral amino acid therapy has been increasingly resorted to in clinical practice. As a rule, hydrolysates of protein, predominantly casein are used. The hydrolysates are made through digestion — either with strong mineral acids or with enzymes. The enzymatic digestion is used, as a rule, because of the disadvantage attaching to the acid hydrolysis (SAHYUN 1944). Enzymatic hydrolysis can be carried out with trypsine and erepsine. When using enzymatic hydrolysis there is, however, always a certain risk for the growth of micro-organisms, which may give rise to pyrogenic substances. Furthermore, decarboxylation may occur and form biogenic amines, which have a pronounced effect on the blood-pressure. To exclude the presence of these substances, a blood-pressure test should always be a part of the control of amino-acid preparations.

WINBURY and CRITTENDEN (1947) have shown that lysine and arginine have a depressive effect on the blood-pressure. About the other amino acids practically nothing is known in this respect.

The aim of the following experiments is to throw light on the effect of the enzymatic casein digest on the blood-pressure.

Experimental.

Preparation. In our experiments we have used a dialyzed, enzymatic casein hydrolysate, named Aminosol[1] (WRETLIND 1947). It contains

[1] Aminosol is prepared by Vitrum, Stockholm.

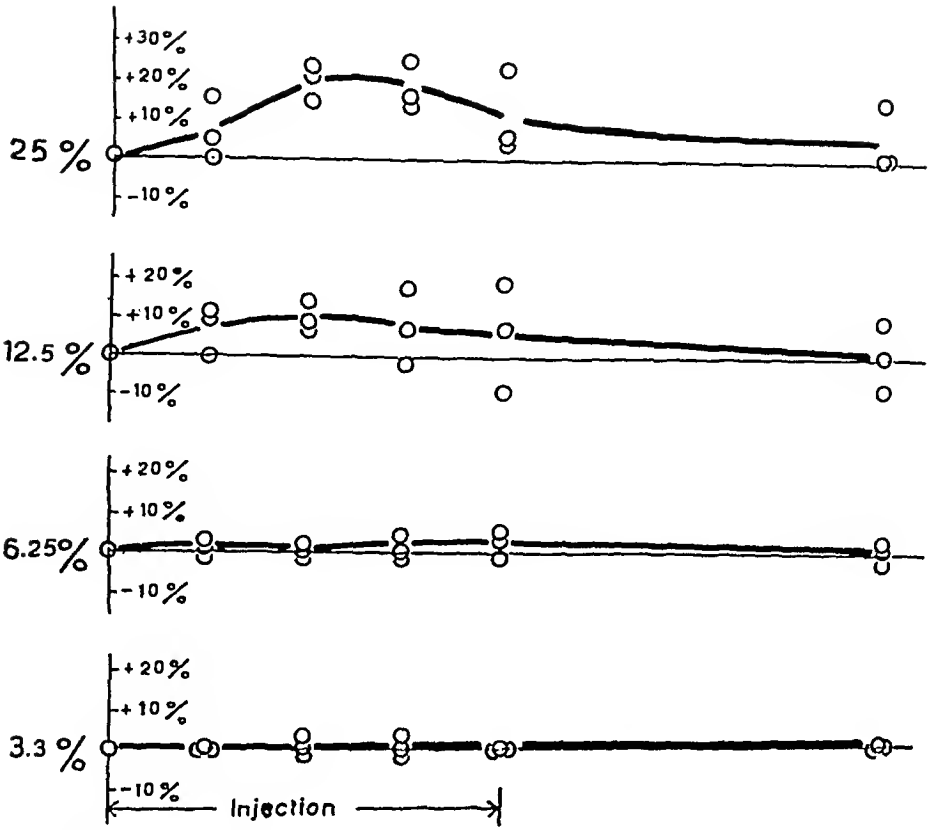

Fig. 1. The effect on the blood-pressure of amino acids of different concentrations.

The abscissa shows the injection time. The ordinata gives the percentual alteration of the blood-pressure. The figures to the left of the ordinata indicate the various concentrations of the amino acids used.

67 % of free amino acids. The remaining percentage consists of low-molecular dialyzable peptides. The original 25 % aminosol solution has a pH of 6.9. To get the various concentrations it was diluted with sterilized distilled water.

Method. Rabbits (1.6—2.8 kg) and cats (2.5—3 kg) were used. The rabbits were anesthetized by intravenous injection of urethane (1.4 g per kg bodyweight). The cats got 0.07 g per kg body-weight of chloralose. A tracheal cannula was inserted and the blood-pressure from the common carotid artery was recorded on smoked paper by means of a mercury manometer. The respiration was registered by a stethograph.

The aminosol solution was injected intravenously with an infusion pump according to LINDGREN (1943). The injection rate was 2.5± ±0.06 ml per minute. The animals got 5 ml of the solutions per kg body-weight. The following concentrations were used: 25, 12.5, 6.25, and 3.3 %. Each concentration was tested on three rabbits. The effect on the blood-pressure is shown in fig. 1. To construct the diagram, we have measured the percentual alterations in blood-pressure three times during

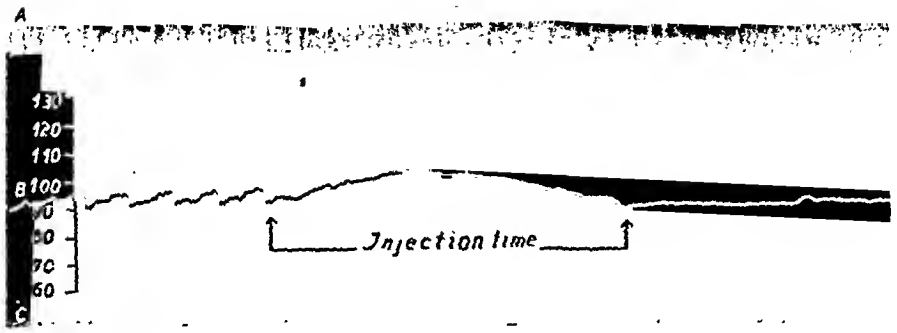

Fig. 2. The effect of a 25 % amino-acid solution on the Traube-Hering-Mayer's waves.

Rabbit weighing 2.1 kg. A = respiration. B = blood-pressure. C = time in 10-second intervals. Before the injection typical Traube-Hering-Mayer's waves. These disappear during the injection of a 25 % amino-acid solution at a rate of 2.5 ml per min. The effect lasts about 30 minutes. Note the simultaneous normalisation of the respiration.

the injection, immediately afterwards, and after double the injection time.

The rate of respiration was measured for a minute immediately before the infusion and for a minute immediately before it came to an end. Table 1 shows the values of respiratory frequencies obtained in the tests on rabbits.

Table 1.

Concentration of aminosol solution	Respiration rate per minute	
	Before injection	During injection
25 %	57	69
12.5 %	80	98
6.25%	40	43
3.3 %	73	74

As rabbits are comparatively insensitive to histamine, the blood-pressure tests were also carried out on cats. The results obtained were similar to those obtained in rabbits.

Results. It emerges from fig. 1, that Aminosol of high concentrations (25 and 12.5 %) undoubtedly raises the blood-pressure of rabbits. Lower concentrations (6.25 and 3.3 %), on the contrary, have little or no influence on the blood-pressure. The maximal rise of blood-pressure, with 12.5 and 25 % solutions, were 10 and 20 % respectively of the original value.

The respiration frequency rose during the injection of Aminosol (table 1). The higher the concentration, the more pronounced was the rise of the respiration rate.

From the blood-pressure tests on cats it could be established, that the amount of histamine, if any, must be too small to have any effect on the blood-pressure.

It was found that injection of a 25 % Aminosol solution on rabbits showing typical Traube-Hering-Mayer's waves made the waves disappear. They returned, however, within 30 minutes. After another injection of 25 % Aminosol they could be made to disappear again. Fig. 2 shows the effect on the Traube-Hering-Mayer's waves.

Discussion.

The above-mentioned experiments by WINBURY and CRITTENDEN (l. c.) prove that in high doses (375 mg per kg bodyweight) the basic amino acids arginine and lysine show a depressive effect on the blood-pressure. At the highest concentration of the Aminosol solutions used about 40 mg of arginine and 75 mg of lysine were given per kg body-weight, amounts too small to give any blood-pressure alterations. In the above experiments it has been shown that a 3.3 % Aminosol solution, the concentration generally used in Scandinavia, exercises no effect on the blood-pressure. If one may assume, that man stands the preparation as well as the animals here used, it should be possible to give 700 ml of a 3.3 % solution in four minutes without any change in blood-pressure. In order to make the body use up all the amino acids, no more than 15 g an hour should be given. If more than 15 g is injected, there is a risk of excretion with the urine. 15 g is the equivalent of 455 ml of a 3.3 % solution. The injection rate in the experiments performed on animals is consequently 23 times as fast. Thus, the safety margin as regards blood-pressure changes is considered to be sufficient. Furthermore, the same preparations has been injected in man at the rate of 1—2 l. of a 3.3 % solution per hour, no secondary reactions being observed.

It has been suggested earlier that a blood-pressure test should always be included in the control of preparations for intravenous use. It may be carried out according to the method mentioned above, the safety margin being considered quite sufficient.

Summary.

Tests have been performed on rabbits and cats, the aim being to investigate the effect of an enzymatic, dialyzed casein digest on blood-pressure. 2.5 ml per minute have been given in concentrations of 3.3, 6.25, 12.5 and 25 %, in all 5 ml per kg bodyweight. A marked rise in blood-pressure followed the injections of 12.5 and 25 % solutions. The 3.3 and 6.25 % solutions have little or no effect on the blood-pressure.

If one may assume that man stands the preparation as well as the animals used, injections of 700 ml of the casein digest (3.3 %) might be given to man in the course of four minutes without giving rise to any blood-pressure effects.

An injection of a 25 % solution of the preparation caused the Traube-Hering-Mayer's waves in rabbits to disappear.

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Phosphorylation and Adenine Nucleotide Uptake of Actomyosin and Actin-free Myosin.

By

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The interaction of adenosine triphosphate (ATP) and actomyosin results in a definite change in the physical properties of the protein. This change has been demonstrated by measurements of the viscosity and flow-birefringence of actomyosin solutions as well as by the volume constriction and the study of the mechanical and optical properties of actomyosin threads (NEEDHAM et al. (1941, 1942), SZENT-GÖYRGYI et al. (1945, 1947), BUCHTHAL et al. (1947)).

Very little is known, however, of any chemical changes in the protein molecule under the influence of ATP. On theoretical grounds, phosphorylation of the protein has been suggested (KALCKAR 1941, DAINTY et al. 1944), while certain experimental findings have led to the postulation of an association of ATP with myosin (SZENT-GYÖRGYI (1945, 1947), MOMMAERTS (1942, 1948)).

In the present investigation it was intended to study the reaction between ATP and actomyosin from the point of view of possible chemical changes in the protein molecule, corresponding to the observed physical effects.

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The experiments were performed on threads of actomyosin or actin-free myosin. The quantitative determination of volume constriction served as a measure of physical changes. Certain aspects of the reaction, especially its correlation with enzymic activity, were investigated on actomyosin solutions.

Method.

Actomyosin solutions: For the investigation of actomyosin solutions the method of ultrafiltration described by BRANDT REHBERG (1943) was used. Equilibrium between inner fluid and ultrafiltrate with regard to dialyzable substances was obtained by low-temperature centrifugation in commercially made cellophane tubes of uniform permeability.

6 ml of an actomyosin solution (dry weight approx. 0.1 per cent) buffered with glycine buffer was heated to 37° C. After addition of 3 ml of the substrate: sodium ATP ((1—4) $\times$ 10^{-6} mol/ml) or sodium dihydrogen phosphate ((5—40) $\times$ 10^{-6} mol/ml) the test tubes were incubated for 30 minutes at 37° C. Experiments were performed at pH 9.1 and a few at pH 7.0. After incubation the tubes were cooled to 0° C., transferred to the cellophane tubes and centrifuged for 5—6 hours at 0° C. (3,000 r. p. m.). After centrifugation they were left to stand overnight at 0° C.

The ultrafiltrate was analyzed directly for phosphorus. 3 ml of the inner fluid was denatured with 1 ml 6 per cent trichloroacetic acid, filtered and analyzed. In two different preparations the adenine contents of the inner fluid and ultrafiltrate were determined.

Threads of actomyosin and actin-free myosin: 5—6 g of threads were expelled into 10 ml distilled water in a centrifuge tube and weighed. A sufficient amount of threads could quickly be obtained by forcing the protein solution (dry weight 3—3.5 per cent) by means of compressed air through the narrow opening of a 10 ml pipette. Before adding the phosphate compound the tubes were kept for 20 minutes at 0° or 37° C. In most experiments 2×10^{-6} moles of the phosphate compound in question were added and after thorough stirring the mixture was allowed to stand for 30 minutes. Generally, experiments were performed with a series of 16 tubes, 8 at 0° and 8 at 37° C., using threads from the same preparation. Two samples from each group of tubes were used as controls without addition of the phosphate compound, but otherwise treated as the others. After high-speed centrifugation for 15 minutes at 2—7° C, the supernatant was decanted and the tube rinsed twice with 25 ml 0.04 M potassium chloride, care being taken not to stir up the sediment. The sediment was then washed by thorough mixing with 20—25 ml 0.04 M potassium chloride, followed by high-speed centrifugation. This procedure was repeated 12 times. In a series of experiments in which the number of washings was varied, *identical results were found after 7 and more washings performed as described.*

After the addition of 5 ml 10 per cent perchloric acid the sediment was ground in a mortar, and the mortar and pestle were washed twice with 1 ml distilled water. Extract and washing water were united, centrifuged at high speed for 15 minutes and filtered. The volume was adjusted to 10 ml and the solution kept at — 18° C. Perchloric acid was chosen as the denaturing agent, as it does not interfere with the spectrophotometric assay of adenine.

Measurement of volume changes in actomyosin threads: The volume constriction produced by ATP was measured for the threads used in each experiment, the same ATP preparation being employed in the same concentration. Measurements were performed microscopically (BUCHTHAL et al. 1947) at room temperature. At low temperature (1° C.) the volume constriction proceeds more slowly and its maximal value is reduced by 25 per cent.

Analytical procedures.

1. Phosphorus was determined by the method of FISKE and SUBBAROW (1925) in the modification of SCHEEL (1936) adapted to a photoelectric colorimeter. Inorganic phosphate (P_0), pyrophosphate (P_7—P_0) and total phosphate (P_T) were determined in all samples, including the controls.

P_0 was determined in 2 ml of the perchloric acid extract after neutralization with concentrated ammonia, using γ-dinitrophenol as indicator.

P_7 was determined in another 2 ml of the extract after 7 minutes hydrolysis in N hydrochloric acid at 100° C and subsequent neutralization as above.

For the P_T determination 4 drops of conc. sulphuric acid and 2 drops of conc. nitric acid were added to 2 ml of the perchloric acid extract and the mixture was heated in a micro-Kjeldahl flask. To avoid splashing a few small folded platinum foils were added. After combustion, cooling and addition of 2 ml distilled water, the solution was boiled for a few minutes, cooled again and neutralized.

After neutralization the reagents were added and the colour intensity measured with a Coleman spectrophotometer at a wave-length of 6,500 Å. The neutralization must be performed very accurately as the colour intensity is highly sensitive to even small variations in the acidity.

Extinction values were converted into absolute phosphate values on the basis of a linear calibration curve obtained with different dilutions of a gravimetrically analyzed solution of sodium dihydrogen phosphate. A content of 3 μg phosphorus in 10 identical samples could be determined with a standard deviation of 3 per cent.

2. Adenine. 2 ml phosphate buffer (pH 7.0) and 0.25 ml N sodium hydroxide were added to 1 ml of the perchloric acid extract (final perchloric acid content < 2 per cent). The adenine content was determined by measuring the absorption maximum at 2,590 Å. A Beckman universal spectrophotometer (model D U) was used and readings taken at 2,400, 2,500, 2,530, 2,560, 2,590, 2,620, 2,650, 2,700 and 2,800 Å.

In evaluating the absolute amounts of adenine, extinction values at 2,590 Å were referred to a calibration curve obtained by measuring the absorption of different amounts of ATP corresponding to 1.6—13 μg of adenine. In order to provide conditions comparable to those in the analyzed sample, different amounts of a standard ATP solution were added to a constant volume (1 ml) of a perchloric acid extract of actomyosin together with phosphate buffer and sodium hydroxide as above. Readings were taken against the same extract without ATP. Identical calibration curves were obtained with four different perchloric acid extracts of actomyosin and different sodium ATP preparations.

3. Ribose. Determination of ribose was carried out according to MEJBAUM (1939) using a Coleman spectrophotometer for the colour measurements at 6,100 Å. The method is very simple and applied to pure ribose or riboside it gave accurate and reproducible values. When it was applied to nucleotides, adenylic acid, adenosine diphosphate and ATP, although containing the same amount of ribose, gave different curves. ATP and adenosine disphosphate gave about double the extinction of pure ribose, and extinction values of adenylic acid lay between those for ATP and ribose. In view of a recent investigation (ALBAUM and UMBREIT (1947)) these difficulties might be overcome by increasing the time of heating of the sample with the reagent. However, when a perchloric acid extract of myosin was added to ATP, the extinction was still higher than that found with ATP alone. Unfortunately different actomyosin extracts gave different calibration curves. How far these differences can be eliminated by changes in the analytical procedure is a matter under investigation. The values for ribose obtained in our experiments will at present be used only for a qualitative estimation.

Substances.

Actomyosin was prepared as described in a previous paper (BUCHTHAL et al. 1947), »actin-free» myosin (SZENT-GYÖRGYI's "crystalline" myosin) according to SZENT-GYÖRGYI (1945).

The preparation of an ATPase-free actomyosin according to SINGHER and MEISTER (1945) did not give uniform results. Although ATPase activity was always considerably reduced, in the present investigations it proved extremely difficult to obtain preparations entirely free fom enzymic activity. Some improvement was obtained by lowering the pH of the buffer solution used for precipitation to pH 5.5.

Adenosine triphosphate was prepared according to the method of NEEDHAM (1942), adapted to large amounts of muscle.[1] Immediately after the slaughter of the animal (ox) 10 kg of muscle was cut out, minced in an electric mincer and extracted with ice-cold 10 per cent trichloroacetic acid. Not more then 5 minutes elapsed from the death

[1] Our thanks are due to chief veterinary TH. ISAKSEN for placing the facilities of the Copenhagen slaughterhouse at our disposal.

of the animal to the immersion of the minced muscle into trichloroacetic acid. After extraction the mixture was squeezed through a cloth and the residue pressed in a hydraulic press. Extraction was repeated with 4 per cent trichloroacetic acid. The combined extracts were sucked through a Buchner funnel containing "Hy Flo" filter mass. A clear solution was obtained, which after neutralization with sodium hydroxide was worked up in the conventional way. The yield was 1.5—3.0 g Barium ATP per kg muscle. Analysis $\frac{P}{N} = 1.33$ (calc. for $C_{10}H_{12}O_{13}N_5P_3Ba_2, 6H_2O = 1.33$).

Adenosine diphosphate was prepared as described by BAILEY (1942). Analysis of a sample gave $\frac{P_7}{P_T} = 0.49$.

Inosine triphosphate was prepared as described by KLEINZELLER (1942). Its purity was estimated by phosphate analysis and by ultraviolet absorption. Analysis of a sample gave an absorption maximum at 2,500 Å and $\frac{P_7}{P_T} = 0.67$.

5-Adenylic acid, inorganic triphosphate, pyrophosphate and orthophosphate were of the same purity as previously used (BUCHTHAL et al. 1944, 1946). All substances were employed as sodium salts.

Results.

1. Actomyosin.

Analysis of threads from thrice-precipitated actomyosin *before addition of ATP* showed in all experiments significant contents of phosphorus, adenine and ribose (Table 1, Fig. 1a). The values given refer to the quantities extractable after denaturation. Previous estimation of the phosphorus content of myosin (BATE-SMITH 1938, BAILEY 1942, LAJTHA 1948) gave considerably higher values (0.04—0.06 per cent) in which, however, the non-extractable fraction is included.

As seen from Table 1, phosphorus appears as inorganic phosphate, pyrophosphate and difficultly hydrolysable organic phosphate. No appreciable difference was found between threads kept at 0° and 37° C. The standard deviation given at the bottom of the table is here slightly higher than in the controls: it covers both uncertainties in the analysis and individual differences in the different protein samples.

1.65 moles of total phosphate were present for each mole of adenine. Of the phosphorus 30 per cent was found as orthophosphate, 30 per cent as pyrophosphate and 40 per cent as difficultly hydrolysable organic phosphate. The ribose content

Table 1.

Contents of phosphate and adenine in untreated actomyosin threads (μg/g).

Actomyosin preparation No.	Temperature 0° C						Temperature 37° C					
	P_0	P_7	P_T	P_7-P_0	P_T-P_7	A	P_0	P_7	P_T	P_7-P_0	P_T-P_7	A
24	0.85	2.15	3.85	1.30	1.70	14.30						
	1.65	2.05	4.30	0.40	2.25	12.20						
25	1.50	2.20	4.05	0.70	1.85	13.10	0.70	1.85	4.35	1.15	2.50	8.40
	1.30	1.70	5.00	0.40	3.30	10.70	0.75	2.70	3.50	1.95	0.80	9.60
27	1.10	2.35	4.45	1.25	2.10	10.85	1.60	2.30	4.90	0.70	2.60	10.70
	1.00	1.90	5.20	0.90	3.30	12.30	1.05	2.15	6.05	1.10	3.90	11.35
	1.10	2.65	4.60	1.55	1.95	13.30						
29	1.45	2.60	4.25	1.15	1.65	11.55	1.30	3.40	4.60	2.10	1.20	10.20
	2.70	3.70	4.90	1.00	1.20	10.90	1.10	2.55	3.80	1.45	1.25	8.65
	1.20	2.85	4.60	1.65	1.75	10.90	0.85	2.40	3.80	1.55	1.40	9.95
	1.05	2.60	3.90	1.55	1.30	9.15	0.95	2.35	3.55	1.40	1.20	10.70
	1.25	2.70	3.50	1.45	0.80	9.80	1.55	3.15	4.00	1.60	0.85	9.85
	1.85	2.80	4.60	0.95	1.80	10.90	1.05	2.35	3.65	1.30	1.30	9.35
	0.90	2.35	4.05	1.45	1.70	9.95						
	1.20	2.40	3.90	1.20	1.50	10.30						
30	1.05	2.40	4.00	1.35	1.60	10.30						
	1.30	2.40	4.15	1.10	1.75	10.00						
	1.25	2.60	3.40	1.35	0.80	10.60						
32	*0.75*	*1.80*	*4.85*	*1.05*	*2.05*	*10.85*	*0.65*	*1.95*	*4.50*	*1.30*	*2.55*	*10.85*
33							1.10	2.30	3.60	2.20	1.30	9.80
							0.45	1.35	3.50	0.90	2.15	7.25
							0.60	1.65	4.10	1.05	2.45	10.30
41	0.45	1.15	3.25	0.70	2.10	15.90	0.70	1.25	3.30	0.55	2.05	16.15
42	1.05	3.05	4.45	2.00	1.40	11.40						
	0.75	1.70	3.00	0.95	1.30	10.35						
	1.20	2.35	4.20	1.15	1.85	11.35	0.95	2.25	4.10	1.30	1.85	10.20
Standard deviation of the observation in per cent	8.8	6.4	4.3			14.1	11.0	8.4	5.5			19.1

was of the same magnitude as that of adenine, but owing to reasons already stated, quantitative data cannot be given.

After addition of ATP (2×10^{-6} mol/ml) considerably higher values were found for adenine (Fig. 1, Curve d) as well as for the different phosphate fractions. The values obtained in experiments on myosin threads (Table 1, 2, 3, 4, 5 and 7) all refer to experiments in which the *washing procedure* as described on p. 327 was repeated

ten to twelve times. A systematic investigation of the phosphate, adenine and ribose contents after one, five, seven, ten and twelve times washing, which was carried out in several experiments, has

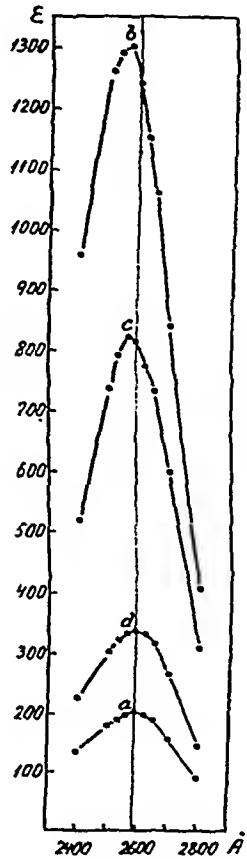

Fig. 1. Ultraviolet absorption of untreated actomyosin (curve a), ATP-treated actomyosin after one washing (curve b), after 5 washings (curve c) and after 7 washings (curve d). With further washing the adenine content remains unaltered.

Ordinates: Extinction in relative units.

Abscissae: Wave-length in Ångström units.

shown that *constant values were obtained after about five to seven times washing*.

Examples of two experiments with two different actomyosin preparations washed 12 times after application of ATP are given in Table 2. At *0° C* on the average 2.35 moles of the total phosphate corresponded to 1 mole of adenine. 35 per cent of the total phosphorus was found as orthophosphate, 35 per cent as pyrophosphate and 30 per cent as difficultly hydrolysable organic phosphate.

In experiments performed at *37° C* with the same actomyosin preparation the total phosphate content was increased by approximately 65 per cent as compared with the values found at 0° C, but no significant increase in the adenine values was observed.

Table 2.

Contents of phosphate and adenine in actomyosin threads (μg/g).

Actomyosin preparation No.	Substances applied	Temperature 0° C						Temperature 37° C					
		P_0	P_7	P_T	P_7-P_0	P_T-P_7	A	P_0	P_7	P_T	P_7-P_0	P_T-P_7	A
27	H_2O	1.10	2.35	4.45	1.25	2.10	10.85	1.60	2.30	4.90	0.70	2.60	10.70
		1.00	1.90	5.20	0.90	3.30	12.30	1.05	2.15	6.05	1.10	3.90	11.35
		1.10	2.65	4.60	1.55	1.95	13.30						
	ATP	3.00	4.65	7.50	1.65	2.85	21.10	6.50	8.75	15.25	2.25	6.50	22.30
		3.30	5.15	8.25	1.85	3.10	19.55	6.30	9.05	14.00	2.75	4.95	16.05
		2.95	5.25	7.75	2.30	2.60	14.60	5.60	7.90	14.10	2.30	6.20	19.35
								5.95	9.00	13.50	3.05	4.50	16.25
								6.45	8.95	14.00	2.50	5.05	16.95
42	H_2O	1.05	3.05	4.45	2.00	1.40	11.40						
		0.75	1.70	3.00	0.95	1.30	10.35						
	ATP	3.15	6.50	8.75	3.45	2.25	14.35	4.20	9.60	11.70	5.40	2.10	18.30
		2.25	4.50	6.75	2.25	2.25	12.65	3.40	8.85	10.50	5.45	1.65	15.05
		2.50	5.25	7.40	2.75	2.15	9.40	4.25	8.30	10.15	4.05	1.85	14.85
		2.50	5.25	7.40	2.75	2.15	9.50	3.70	8.20	10.50	4.50	2.30	16.50
		2.95	6.25	8.15	3.30	1.90	12.80	4.60	8.50	11.25	3.90	2.75	16.50
		2.95	6.50	8.45	3.55	1.95	15.80	3.55	7.45	9.85	3.90	2.40	16.30
								3.95	8.85	11.50	4.90	2.65	17.10
								4.20	9.15	10.75	4.95	1.60	16.30

The increase was most pronounced for orthophosphate (80 per cent) and amounted to 55 per cent for both pyrophosphate and difficultly hydrolysable organic phosphate. The ribose content was also increased at 0° and 37° C as compared with that of untreated threads.

After application of ATP the *volume constriction* of the threads determined on a parallel sample amounted on the average to 70 per cent (20° C).

The ATP concentration employed (2.0×10^{-6} mol/ml) corresponded approximately to that causing maximal volume constriction. In order to investigate whether the observed chemical effects likewise reached maximal values, the influence of increasing amounts of ATP was studied. As seen from Fig. 2, the values of pyrophosphate, orthophosphate and adenine increased at approximately the same rate over the range of the ATP concentrations investigated, while the difficultly hydrolysable organic phosphate content remained unchanged.

When ATP was substituted by equimolar amounts of adenosine diphosphate, adenylic acid or inosine triphosphate *no difference* was found in either the purine or the phosphate content of actomyosin before and after treatment with these compounds. Likewise the actomyosin remained unchanged after treatment

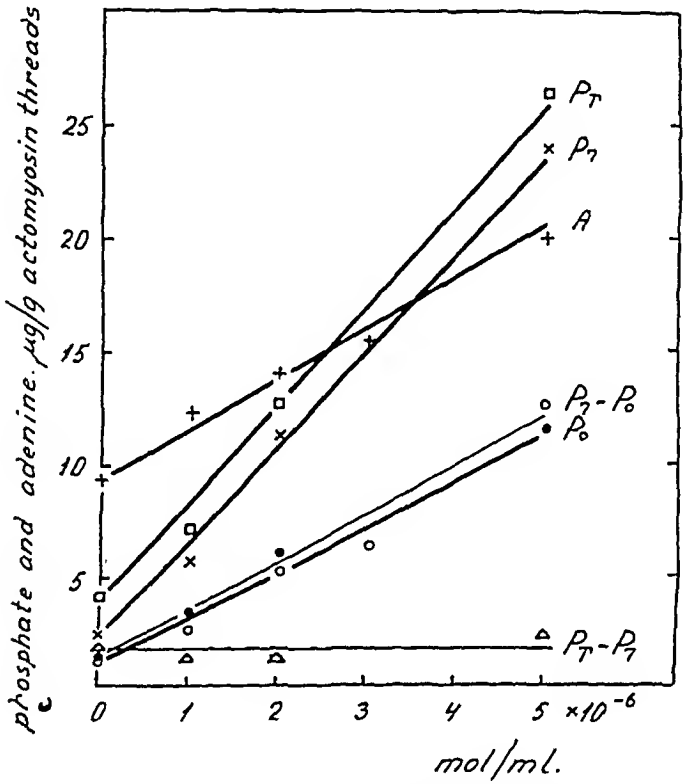

Fig. 2. Phosphate and adenine uptake by actomyosin threads with increasing ATP concentrations (37° C).
Ordinates: Phosphate and adenine content in µg per g. threads.
Abscissae: ATP concentrations in mol/ml.

with even four times larger quantities (8×10^{-6} mol/ml) of orthophosphate, pyrophosphate or triphosphate (Table 3). No volume constriction was associated with the application of these substances.

2. Actin-free myosin.

For orthophosphate, pyrophosphate and adenine approximately the same values were obtained with *untreated threads* of actin-free myosin as with untreated actomyosin, whereas the total phosphorus content was about 50 per cent lower. Identical

Table 3.

Contents of phosphate and adenine in actomyosin threads (μg/g).

Actomyosin preparation No.	Substances applied	Temperature 0° C						Temperature 37° C					
		P_0	P_7	P_T	P_7-P_0	P_T-P_7	A	P_0	P_7	P_T	P_7-P_0	P_T-P_7	A
30	H_2O	1.05	2.40	4.00	1.35	1.60	10.30						
		1.30	2.40	4.15	1.10	1.75	10.00						
		1.25	2.60	3.40	1.35	0.80	10.60						
	Adenosine diphospate	1.35	2.40	4.50	1.05	2.10	10.65						
		1.45	2.70	4.90	1.25	2.20	11.80						
		1.15	2.90	5.35	1.75	2.45	12.40						
		1.55	2.80	5.30	1.25	2.50	11.50						
		1.40	3.00	5.80	1.60	2.80	12.20						
29	H_2O	1.45	2.60	4.25	1.15	1.65	11.60	1.30	3.40	4.60	1.10	1.20	10.20
		2.70	3.70	4.90	1.00	1.20	10.90	1.10	2.55	3.80	1.45	1.25	8.65
		1.20	2.85	4.60	1.65	1.75	10.90						
	Adenylic acid	1.05	2.60	3.90	1.55	1.30	9.15	0.85	2.40	3.80	1.55	1.40	10.00
		1.25	2.70	3.50	1.45	0.80	9.80	0.95	2.35	3.55	1.40	1.20	10.70
		1.85	2.80	4.60	0.95	1.80	10.90	1.55	3.15	4.00	1.60	0.85	9.85
		0.90	2.35	4.05	1.45	1.70	10.00	0.90	2.50	3.90	1.60	1.40	
		1.20	2.40	3.90	1.20	1.50	10.30	1.05	2.35	3.65	1.30	1.30	9.35
41	H_2O	0.45	1.15	3.25	0.70	2.10	15.95						
	Inosine triphosphate	0.55	2.45	2.60	1.90	0.15	16.30						
		0.75	2.40	4.90	1.65	2.50	14.95						
29	H_2O	1.10	2.15	3.70	1.05	1.55							
		1.00	2.15	3.90	1.15	1.75							
	Orthophosphate	1.70	1.85	2.40	0.15	0.55							
		1.40	1.90	2.15	0.50	0.25							
	Pyrophosphate	1.20	1.55	2.05	0.35	0.50							
		1.05	1.05	1.30		0.25							
	Triphosphate	0.75	1.55	2.70	0.80	1.15							
		1.05	1.30	1.50	0.25	0.20							

results were obtained for threads kept at 0° and 37° C, just as in the case of untreated actomyosin (Table 4).

Addition of ATP (2×10^{-6} mol/ml) resulted in a remarkably high uptake of all phosphate fractions, as well as of adenine and ribose at 0° C. 3.3 moles of total phosphorus corresponded to 1 mole of adenine. 45 per cent of the total phosphorus was found as orthophosphate, 20 per cent as pyrophosphate and 35 per cent as difficultly hydrolysable organic phosphate. This distribution of

Table 4.

ntents of phosphate and adenine in actin-free myosin threads (μg/g).

Actin-free myosin preparation No.	Substance applied	Temperature 0° C						Temperature 37° C					
		P_0	P_7	P_T	P_7-P_0	P_T-P_7	A	P_0	P_7	P_T	P_7-P_0	P_T-P_7	A
20	H_2O	0.80	0.85	1.45	0.05	0.60	6.95						
		0.65	1.00	1.30	0.35	0.30	7.35						
	ATP	5.55	8.85	14.40	3.30	5.55	16.50	10.70	17.00	25.30	6.30	8.30	21.70
		6.85	10.40	16.70	3.55	6.30	18.55	12.10	18.50	26.20	6.40	7.70	25.00
		5.55	9.10	14.20	3.55	5.10	18.00						
		5.80	9.20	13.30	3.40	4.10	18.70						
28	H_2O	0.95	1.80	3.40	0.85	1.60	12.65	1.25	1.60	3.40	0.35	1.80	13.20
	ATP	6.20	8.75	17.50	2.55	8.75	23.50	6.80	7.20	12.90	0.40	5.70	21.90
		5.20	7.15	11.50	1.90	4.35	23.10	11.25	12.80	17.50	1.55	4.70	26.80
		5.80	8.15	14.50	2.35	6.35	25.20	7.60	8.70	14.20	1.10	5.50	29.60
31	H_2O	2.20	3.90	5.20	1.70	1.30	9.95	1.90	3.10	4.50	1.20	1.40	8.25
	ATP	7.00	10.90	12.70	3.90	1.80	12.65	7.10	10.90	13.40	3.80	2.50	12.60
		6.70	9.80	11.80	3.10	2.00	12.10	8.20	12.00	14.50	3.80	2.50	13.10
		7.80	11.10	12.90	3.30	1.80	13.00	8.40	11.20	13.50	2.80	2.30	11.75

phosphate fractions was approximately the same as in the untreated threads.

At 37° C the nucleotide uptake was practically identical with that at 0° C and the increase in total phosphorus could be accounted for by orthophosphate alone (Table 4).

3. Actomyosin with low triphosphatase activity.

The phosphate content of the threads *before addition of ATP* corresponded approximately to that of actomyosin which was not treated, according to SINGHER and MEISTER (1945) (Table 5).

Treatment with ATP (2×10^{-6} mol/ml) at 0° C resulted in an uptake of total phosphate corresponding to more than three times the total phosphate content of the untreated protein. The ribose content was likewise increased, while adenine could not be estimated owing to a slight contamination of the protein with veronal. 40 per cent of the total phosphate content appeared as orthophosphate, 15 per cent as pyrophosphate and 45 per cent as difficultly hydrolysable organic phosphate. The addi-

Table 5.

Contents of phosphate in actomyosin threads with low ATPase activity (μg/g).

Actomyosin preparation No.	Substance applied	Temperature 0° C					Temperature 37° C				
		P_0	P_7	P_T	P_7-P_0	P_T-P_7	P_0	P_7	P_T	P_7-P_0	P_T-P_7
23	H_2O	1.40	2.15	4.00	0.75	1.85					
	ATP	6.00	8.60	15.30	2.60	6.70	7.10	8.25	15.60	1.15	7.35
		4.95	7.15	9.80	2.20	2.65	6.40	9.70	17.90	3.30	8.20
	ATP + KCl + $MgCl_2$	10.95	12.00	18.70	1.05	6.70					
		11.95	14.20	22.10	2.25	7.90					

tional phosphate uptake at 37° was approximately 20 per cent (Table 5). In this table results from preliminary experiments are given which indicate that the phosphate uptake is enhanced in the presence of magnesium chloride (0.01 M) and potassium chloride (0.1 M) which, according to SZENT-GYÖRGYI (1941—42) and our own observations, also enhance volume constriction.

Volume constriction, as shown in a previous paper (BUCHTHAL et al. 1947), is approximately the same in enzymically active and inactive actomyosin threads.

Ultrafiltration experiments.

Estimation of orthophosphate in the actomyosin solutions (dry weight c. 0.2 mg/ml) after ultrafiltration *without addition of ATP* gave values in the inner fluid of 0.5—1.0 μg/ml, *i. e.* 2—3 times more orthophosphate per mg protein than in the thread experiments. This difference is possibly due to the repeated washing in the latter experiments.

After addition of ATP ($(1.0—4.0) \times 10^{-6}$ mol/ml) in experiments performed at 37° C, higher phosphate values were found in the inner fluid, increasing with increasing ATP concentrations. At 0° C the changes observed were within the limits of experimental error. The curve in Fig. 3 represents experiments with different actomyosin preparations with or without enzymic activity at 37° C. The phosphate uptake per g protein exceeded that found in actomyosin threads. It must, however, be borne in mind that the ATP concentration relative to the actomyosin concentration was 15 times higher, since the actomyosin content of the solutions

was 15 times lower than that used in thread experiments. The adenine content in the fluid inside the cellophane bags also exceeded that of the ultrafiltrate.

When ATP was substituted by orthophosphate in higher concentrations ((5.0—40.0) $\times$ 10^{-6} mol/ml), the phosphate content of the inner fluid in the cellophane bags was likewise increased, indicating an uptake of phosphate by the protein, but in contrast

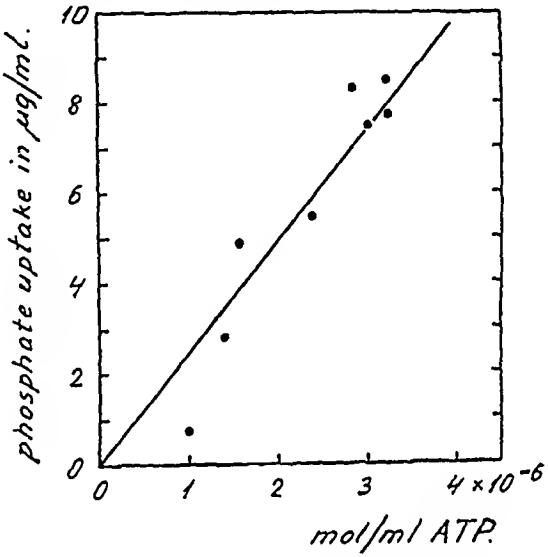

Fig. 3. Phosphate uptake found in inner fluid in ultrafiltration experiments as function of the ATP concentration applied (37° C).
Ordinates: Phosphate in inner fluid in µg/ml.
Abscissae: ATP concentrations in mol/ml.

to ATP no systematic correlation existed between concentration of phosphate applied and taken up (Table 6). The uptake of phosphate showed a considerable variability. Thus, phosphate, when added in relatively large amounts, was adsorbed to actomyosin, an effect which is not confined to this protein, as preliminary experiments with lactoglobulin gave similar results. Since, however, attempts to remove adsorbed phosphate by washing would be difficult to make with the ultrafiltration technique employed, the latter seems hardly suitable to differentiate between true phosphorylation and phosphate adsorption.

SZÖRENYI and CHEPINOGA (1946) come to somewhat different conclusions in a study of the dialysis (24 hours) of actomyosin against a salt solution. A free diffusion of phosphate is claimed, when di-potassium hydrogen phosphate is added in a concentration of 20 $\times$ 10^{-6} mol/ml to an actomyosin solution, for which

Table 6.

Preparation	Orthophosphate added $\times 10^{-6}$ mol/ml	Actomyosin concentration per cent (dry weight)	Orthophosphate		Difference[1] between inner fluid and ultrafiltrate µg/ml	Number of samples
			ultra filtrate µg/ml	inner fluid µg/ml		
Actomyosin ...	5.0	0.1	31	48	16	4
Actomyosin ...	5.0	0.3	31	48	6	3
Actomyosin, low ATPase activity	6.7	0.3	58	74	9	3
Actomyosin ...	6.7	0.3	56	60	4	8
Actomyosin, low ATPase activity	10.0	0.2	84	94	10	7
Actomyosin ...	10.0	0.1	80	93	13	8
Actomyosin ...	10.0	0.6	81	93	12	4
Actomyosin, low ATPase activity	20.0	0.3	110	116	6	2
Actomyosin ...	42.0	—	116	136	19	3

concentration values are not given in the English summary or the tables. In the presence of ATP, phosphate is bound, but from the scanty information available it is difficult to estimate how far differences in the experimental procedure, *i. e.* pH, incomplete dialysis or other factors are responsible for the divergent findings.

Discussion.

It has previously been shown that myosin contains a certain amount of phosphorus (BATE-SMITH 1938, BAILEY 1942, LAJTHA 1948). The phosphorus extractable after denaturation, according to our investigations, is present as readily and difficultly hydrolysable phosphate and also as a highly labile phosphate which splits off by denaturation and appears as orthophosphate in the analysis. In addition, both actomyosin and actin-free myosin contain appreciable amounts of adenine and ribose. Repeated washing reduces the different phosphate fractions and the adenine content by only 10 to 20 per cent. The ratio of the readily and difficultly hydrolysable phosphate to adenine is lower than would be expected if the nucleotide were present as adenosine diphosphate.

After treatment with ATP the phosphate, adenine and ribose contents of the protein are considerably increased. Mean values

[1] The phosphate content of the samples before addition of orthophosphate has been subtracted.

Table 7.

Content in phosphate and adenine (µg/g threads).

	Substances applied	Number of experiments	Temperature 0° C						Number of experiments	Temperature 37° C					
			P_0	P_7	P_T	P_0-P_7	P_T-P_7	A		P_0	P_7	P_T	P_0-P_7	P_T-P_7	A
Actomyosin preparations No. 27, 42	H_2O	22	1.20	2.35	4.20	1.15	1.85	11.35	15	0.95	2.25	4.10	1.30	1.85	10.20
	H_2O	5	1.00	2.35	4.35	1.35	2.00	11.30	2	1.30	2.30	5.55	1.00	3.25	11.00
	ATP	9	2.85	5.50	7.80	2.65	2.35	14.40	13	4.80	8.65	12.10	3.85	3.45	17.05
Actin-free myosin preparations No. 20, 28, 31	H_2O	4	1.15	1.90	2.85	0.75	0.95	9.20	4	1.60	2.35	3.95	0.75	1.60	10.55
	ATP	10	6.25	9.35	13.95	3.10	4.60	18.15	8	9.20	12.30	17.10	3.10	4.80	20.30
Actomyosin with low ATPase activity, preparation No. 23	H_2O	1	1.40	2.15	4.00	0.75	1.85								
	ATP	2	5.50	7.85	12.55	2.35	4.70		2	6.75	9.00	16.75	2.25	7.75	
	ATP+KCl + $MgCl_2$	2	11.45	13.10	20.40	1.65	7.30								

from all the experiments are given in table 7. The relative amounts of readily and difficultly hydrolysable phosphate now correspond roughly to their proportion in adenosine diphosphate. SZENT-GYÖRGYI (1945) on the basis of the acid-hydrolysable phosphate content of ATP-treated myosin postulated a binding of ATP to myosin. The present experiments give no indication of a binding of ATP as such.

Our results refer to the *values obtained after thorough washing* of the ATP-treated protein. Washing was found to cause not only a reduction in phosphate and nucleotide content of the protein, but also a qualitative change in the nucleotide (Fig. 1). ATP-treated protein that is washed only once shows a distinct displacement of its ultraviolet absorption-maximum towards shorter wave-lenghts (2,540 Å), as compared with the maximum found after three to twelve washings or in ATP solutions (2,590 Å). The difference suggests a deamination of free or loosely bound adenine nucleotide reminding of a similar effect described by BANGA and JOSEPOVITS (1947) for ATP solutions.

The amount of adenine nucleotide in actomyosin after treatment with ATP (2×10^{-6} mol/ml) amounts to approximately 2 mg/g actomyosin (dry weight) calculated as ATP, which is one seventh of the ATP normally present in muscle. In all probability the difference is due mainly to the relatively low ATP concentrations used in most of our thread experiments. The adenine-nucleotide content in myosin threads is increased with higher ATP concentrations, and it is also apparent from the values obtained in ultrafiltration experiments, in which higher concentrations were applied, that they approach those found in the muscle fibre.

The most striking result of the present investigation was the *finding that a considerable increase in the orthophosphate fraction occurred after treatment with ATP*. The orthophosphate found before and after treatment with ATP must originate from highly labile phosphate, bound to the protein and appearing as orthophosphate after denaturation. Thus, it must be considered to be the expression of a phosphorylation of the protein. It can hardly be present as orthophosphate before denaturation as control experiments show that orthophosphate added to the protein is quantitatively removed by repeated washings preceding denaturation. The phosphate adsorption found in our ultrafiltration experiments is observed only when much higher concentrations of orthophosphate are applied. Furthermore, in these experiments no

attempts were made ro remove the adsorbed phosphate by washing.

The chemical effects described showed no direct relation to the ATPase activity of the protein. For example, there was no difference in the phosphate and adenine-nucleotide uptake in preparations with high and low ATPase activity. Furthermore, inosine triphosphate, which is more readily split by ATPase than ATP, does not cause any measurable chemical changes in the protein. The same is true for inorganic triphosphate which, according to DAINTY et al. (1944), is likewise split by ATPase. In comparative experiments performed at 0° and 37° C we have found a 40 times higher enzymic activity at 37° C, while the phosphate and nucleotide uptake increases only 1.25 times over the range of temperature investigated.

Finally, the enzymic activity of an actomyosin, previously treated with ATP and washed out, is the same as that of a control preparation untreated with ATP and washed out in the same way. Accordingly, the previous uptake of phosphate and adenine nucleotide does not reduce the enzymic activity of the preparation.[1]

The specificity of ATP in initiating the chemical changes described, brings to mind the high specificity of ATP in releasing volume constriction. In this connection it is of special interest that the application of inosine triphosphate is not accompanied by chemical changes or by volume constriction. An increase in the concentration of ATP increases the volume constriction and the uptake of phosphate and adenine nucleotide (Fig. 2). Addition of compounds such as magnesium salts, which likewise increase the effect of ATP on volume constriction results in an enhanced phosphorylation. According to MOMMAERTS and SERAIDARIAN (1947) magnesium chloride applied under similar conditions, *i. e.* in the presence of potassium chloride, inhibits the enzymic activity of actomyosin.

In several particulars a parallel may be drawn between volume constriction which is initiated by ATP and these chemical changes produced by the nucleotide. However, volume constriction occurs only in the presence of actin. Threads from actin-free myosin do not show volume changes, while the chemical effects are approximately the same for the both preparations. Hence,

[1] These experiments were kindly performed by Dr. and Mrs. *A. Szent-Györgyi jr.* during their stay in this laboratory.

the chemical changes in actomyosin produced on interaction with ATP are localised to the myosin moiety of the protein and occur in the absence of actin as well.

Summary.

1. Actomyosin and actin-free myosin contain adenine (10 μg/g threads), ribose and phosphorus (4 μg/g threads) extractable after denaturation. The phosphorus is present as readily hydrolysable phosphate (1 μg/g threads), difficultly hydrolysable phosphate (1—2 μg/g threads) and in the form of a labile phosphate (1—2 μg/g threads) which appears as orthophosphate after denaturation.

2. Treatment with ATP results in a considerable increase in all the fractions mentioned above, which cannot be removed by washing. The ratio of readily hydrolysable and difficultly hydrolysable phosphate to adenine corresponds roughly to the proportion of these fractions in adenosine diphosphate.

3. The labile phosphate fraction found must be considered as brought about by the phosphorylation of the protein by ATP.

4. The chemical changes are highly specific for ATP. Inosine triphosphate, adenosine diphosphate, adenylic acid, orthophosphate, pyrophosphate and triphosphate in similar or larger amounts are without effect.

5. No direct relationship exists between phosphate and nucleotide uptake on the one hand, and ATPase activity of actomyosin and actin-free myosin on the other, since actomyosin with high or low enzymic activity gives approximately the same effects. The dependence of the enzymic activity on temperature is much higher than that of the chemical effects described.

6. A close correlation between volume changes and chemical changes is indicated by the specificity of ATP for both reactions. Both are increased with increasing amounts of ATP and after the addition of small amounts of magnesium salts. The chemical changes are localised to the myosin part of actomyosin.

7. Ultrafiltration experiments likewise show an uptake of phosphate by an actomyosin solution after treatment with ATP, which increases with increasing ATP concentrations. Orthophosphate, when applied in relatively high concentrations, is more highly concentrated in the inner fluid than in the dialysate, indicating an adsorption of the orthophospate by the protein. No constant relation was found between the amounts of ortho-

phosphate applied and adsorbed, after a certain minimum concentration had been reached.

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The Effect of DFP on Respiration, Blood Pressure and Muscular Function in the Rabbit.

By

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Amongst the war gases which have been developed recently, di-isopropyl-fluorophosphonate (DFP) is no doubt the most interesting one from the pharmacological point of view. It was produced by Mc COMBIE and SAUNDERS (1946), whose attention was drawn to these substances by the investigations of LANGE and KRÜGER (1932) in the matter of dimethyl and diethyl-fluorophosphonate. DFP has proved to inhibit and partly to destroy the enzyme cholin-esterase (MACWORTH, MAZUR and BODANSKY 1946; KOELLE and GILMAN 1946) and to possess both the muscarine and the nicotine effect of acethylcholine (MODELL, KROP, HITCHCOCK and RIKER, 1946; Mc NAMURA, KOELLE and GILMAN, 1946; HORTON, KOELLE, Mc NAMURA and PRATT, 1946; COMROE, TODD and KOELLE, 1946; SCHOLZ, 1946). The cause of death from DFP intoxication is, however, not quite clear. According to most investigators, death is due to paralysis of respiration. COON (1944) holds that it is due to a bronchial-muscular contraction and a contracture of the respiratory muscles. MODELL et al. (l. c.) discuss the cause of death in detail. They hold that it is not caused by a peripheral paralysis of respiration, since the neuromuscular transmission is inhibited only when intoxication is far advanced, but by paralysis of the respiratory and vasomotor centres of the medulla.

In order to arrive at a rational treatment of DFP intoxication, it was deemed desirable to ascertain the cause of death, and this was the chief object of this work. As it was evident both from the literature and from my own preliminary experiments that the cause of death was either a paralysis of respiration or an insufficiency of circulation, the effect on respiration and blood pressure was investigated. As it appeared at an early stage that paralysis of respiration was the probable cause of death, attention was centred chiefly on the effect of DFP on respiration.

Method.

DFP was administered in Ringer solution at a concentration of 0.3—1.0 mg/cc. The solutions were prepared immediately before each test, as DFP is not stable in a water solution (Modell et al., l.c.). Small doses of DFP were generally given repeatedly in order to secure a slow development of intoxication. Blood pressure was recorded with a mercury manometer from the femoral artery in those cases where respiration was specially studied; in other cases from the common carotid artery. Respiration was recorded with a body plethysmograph as modified by v. Euler and Liljestrand (1936), and in the case where the muscular function was recorded, simultaneously with a pneumograph and Marey's capsule. In order to stimulate the diaphragm, the right phrenic nerve was freed without being severed, on a length of 1 cm directly below its exit from the cervical spine. To avoid damage to the nerve, a strip of the fascia of the deep neck musculature was also freed and used as a foundation for the nerve. The stimulation was effected via silver electrodes with an inductorium. The strength and the frequency was adapted so that a maximum tetanic contraction of the diaphragm was secured, with the least effect on the muscles of the legs. The muscular function was examined on the gastrocnemius and tibialis anticus muscle. The femur or the tibia was fastened to a Brown-Schuster stand with a perforating pin, and the muscle tendon connected to an isometric steel spring myograph. The other muscles to the leg were severed. The stimulation was effected both indirectly via the sciatic nerve with silver electrodes and directly with plate electrodes. Stimulation from an inductorium with varying frequencies. The strength of stimulation was supramaximal.

As it proved that the DFP substance produced at the Research Institute for National Defence had not the anticipated activity, its toxicity was determined on 60 white mice, according to Bliss' graphical method (1938). DFP was given in 0.9 % common salt solution by subcutaneous injection and LD^{50} was found to be 42 mg per kg of body weight. Horton, Koelle, Namura and Pratt (l. c.) indicate $LD^{50} = 3.71$ mg/kg of body weight. Thus the activity of the substance at my disposal was 9 % of that of the chemically pure substance. The

same result is arrived at from the calculation of the lethal intravenous dose for rabbits (LD^{100}) which was on an average, 5 mg/kg for 15 animals, whilst the authors referred to above indicate 0.5 mg/kg, which gives a concentration of 10 % of the substance examined. Consequently the activity of the substance at my disposal was only about 10 %. The doses given were computed so that they corresponded with the pure substance.

Results.

The general symptoms of intoxication were investigated on 5 non-anesthetized rabbits and turned out, in conformity with the descriptions of MODELL et al. (l. c.). After the animals had received 0.5—1.0 mg of DFP i. v., they became restless after a few minutes with hurried respiration, fibrillar muscular contraction and discharge of faeces. Gradually a considerable weakness developed in the hind legs. Respiration became slow and irregular and was carried out with the help of the auxiliary respiratory muscles. At the same time the paralysis of the muscles spread proximally, and in the final stage the animals lay almost completely paralysed with constantly increased respiratory difficulties. The local application of DFP to the eyes of the animals caused maximal miosis. In rabbits anesthetized with urethane the symptoms were identical. Especially characteristic features, apart from the changes in respiration and blood pressure described below, are the fibrillar muscular contractions and discharge of faeces. An increased flow of saliva and tears was also observed.

The intoxication picture of mice was characterized by tremor and consequent muscular weakness, respiratory difficulties and sometimes by salivation.

The effect on respiration and blood pressure was examined on 23 rabbits narcotized with urethane, 1.4 g. urethane per kg body weight being used. For the sake of clearness, the effects on respiration and blood pressure are treated separately.

Respiration.

If a large dose of DFP is given, respiration ceases within a minute or two, both the frequency and the amplitude being reduced. The changes set in too quickly for a detailed study to be possible. If repeated small doses of DFP are given, the intoxication develops more slowly, however. Two stages may be distinguished.

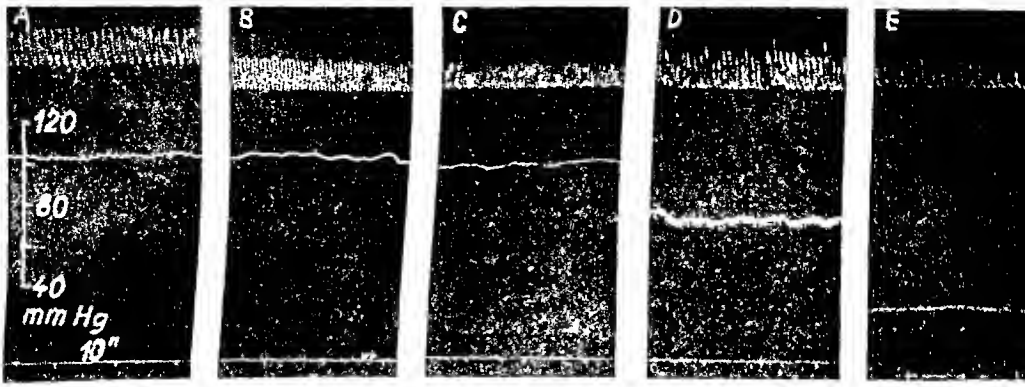

Fig. 1. Test 6. The effect of DFP on respiration and blood pressure.
A. normal respiration and blood pressure at 0^{00}. At 0^{01} 1.6 mg DFP.
B. at 0^{08}. C. at 0^{10}. At 0^{19} further 0.4 mg DFP.
D. at 0^{21}. At 0^{32} 0.8 mg DFP.
E. at 0^{16}.

During the first stage respiration becomes more rapid, whereas the amplitude diminishes (Fig. 1, B and C). If a suitable dose of DFP has been given, this stage is fairly stationary for up to 30 minutes, which renders possible a quantitative study of it. In Table 1 all the experiments on intact rabbits have been collocated. A statistical analysis of the material according to the t-method (FISCHER 1936) is found in Table 4. As shown therein, the frequency increases by 46 % on an average, whereas the volume decreases by 35 % on an average. Both the increase of the frequency and the decrease of the volume are statistically significant. No certain change of the ventilation can be proved.

The first stage of DFP intoxication passes over into the second one, either spontaneously, viz. in those cases where a large dose of DFP has been given, or as a result of a fresh dose. This stage exhibits different features in different rabbits. It is, however, possible to distinguish between two types. With the first type the respiration changes character, the frequency decreasing and the volume increasing (Fig. 1, D). The ventilation, however, decreases. In this stage the animals become cyanotic and use the auxiliary respiratory muscles. Gradually both the volume and the frequency decrease (Fig. 1, E), the animal dying owing to cessation of respiration.

With the second type both the frequency and the volume of each respiration decrease and cessation of respiration sets in quickly. The animal does not use the auxiliary respiratory muscles.

Table 1.

Effect of DFP on the blood pressure and respiration of intact rabbits.

Test No.	Weight of animal kg	Blood pressure and respiration before injection of DFP.				1st stage of the DFP-intoxication. Blood pressure and respiration Increase or decrease per cent.					2nd stage of the DFP-intoxication according to:		
		Blood press. mm Hg	Resp. rate per min.	Volume of each respir. ml	Ventilation ml. per min.	DFP mg	Blood press.	Respiration			DFP mg	Type I	Type II
								Rate	Volume	Ventil.			
1	3.0	116	70	15.8	1092	0.4	0	+ 43	— 26	+ 7	0.6	+	
2	2.1	104	62	11.7	725	1.2	— 8	0	— 29	— 29	1.9		+
3	2.3	110	56	11.7	655	1.0	+ 11	+ 125	— 36	+ 44	2.8	+	
4	2.8	130	43	23.3	1000	1.6	+ 9	+ 33	— 43	— 24	2.3	+	
5	3.0	118	68	12.5	850	1.0	— 15	+ 29	— 26	— 5	1.9		+
6	3.2	102	46	15.8	727	1.6	+ 2	+ 122	— 37	+ 40	2.7	+	
7	2.8	100	72	15.0	1080	0.9	+ 10	+ 39	— 45	— 23	1.4	+	
8	2.2	110	77	13.3	1024	0.9	+ 4	+ 22	— 38	— 24	1.4	+	
9[1]	3.5	90	74	25.0	1850	1.8	0	+ 51	— 33	+ 1	2.5	+	
10[1]	2.7	—	60	27.7	1662	1.3	—	+ 27	— 40	— 24	—	—	—
11[1]	2.8	100	56	20.8	1160	1.6	+ 20	+ 16	— 28	— 16	4.2		+

[1] These animals were vagotomized and the sinus region was denervated after the 1st stage of the DFP-intoxication had developed.

This distinction is somewhat schematic, because the first type sometimes lasts 10—15 minutes, sometimes there are only about ten deep breathings. As the second stage shows a progressive reduction of respiration and is not of a stationary nature, it was impossible to deal with it quantitatively. Therefore in Table 1 only the type of the second stage is given. Type 2 appeared in 2 out of 8 experiments.

The relation between the dose which resulted in the first stage and the one which caused cessation of respiration was 0.65 on an average.

Point of Attack of Respiration.

Respiration can be affected either directly through the respiratory centre or via the carotid and aortic bodies or peripherally through nerves, neuromuscular transmission or muscles.

According to HEYMANS, BOUCKAERT, FARBER and HUS (1936) acetylcholine via the chemoreceptors in the sinus region causes hyperpnea and tachypnea on dogs. SCHWEITZER and WRIGHT (1938) analyzed the effect of acetylcholine on the respiration of the cat. The drug produces a threefold effect, consisting of an initial stimulation, followed by depression and secondary stimulation. The point of attack of this last was the chemoreceptors in the carotid body, whereas the inhibiting influence seemed to be due to a direct effect on the respiratory centre and the spinal cord. In view of the fact that DFP, being a cholinesterase-inhibiting substance, acts like acetylcholine, it was considered to be of interest to examine whether DFP affects respiration via the carotid body.

However, it proved to be combined with certain difficulties to examine the effect on respiration of DFP quite clearly. This was due to the fact that the effect produced by DFP was irreversible — at least within a reasonable time. The denervation of the sinus region had therefore to be carried out whilst the intoxication was in progress, in order to permit of an observation of the effect on respiration before and after denervation of the same animal. However, the denervation lasts for a certain time — about 10 minutes. During that time respiration has had time to change character under the effect of DFP, and an effect, which is attributed to the denervation, may also be due to a progress of the DFP intoxication. In addition, vagotomy must be carried out

to disconnect the aortic body. In order as far as possible to take account of these circumstances, vagotomy was carried out on a group of animals, before DFP was given, and thereafter the carotid bodies were denervated during the first stage of the DFP intoxication. The results are shown in Table 2. Another group was both vagotomized and denervated before DFP was given (Table 3). The denervation was tested by letting the animal breathe 7 % of oxygen in nitrogen. Decreased respiration and falling blood pressure were taken as signs that the denervation had been successful (HEYMANS, BOUCKAERT and DAUTREBANDE, 1930; v. EULER and LILJESTRAND l. c.). As appears from Table 2, denervation of sinus region has no effect on respiration symptoms after DFP. From Table 3 it is also apparent that DFP has the same effect on sinus-denervated animals as on intact ones. In Table 4 the differences between the effect on frequency, volume of respiration and total ventilation are shown for intact animals and sinus denervated ones. In no case is there any indication that the differences are statistically significant.

The second stage of DFP intoxication seems, however, to be influenced by sinus denervation. As the tables show, in 5 cases out of 13 the second stage is of type 1 with the sinus-denervated animals, whereas the corresponding figures for the intact animals are 6 out of 8. The statistical analysis — according to the χ^2-method for heterogeneity (BONNIER-TEDIN 1940) — shows that the probability that this difference is due to chance is 0.05. Thus the difference is not statistically significant but yet probable.

As DFP did not seem to work via the carotid body, an attempt was made to investigate whether the point of attack was situated peripherally. This was done because MODELL et al. (l. c.) have shown that DFP inhibits the neuromuscular transmission.

The study of the general symptoms of intoxication indicated that muscular paralysis starts in the hind legs and then spreads proximally. In view of the fact that different groups of muscles become paralyzed at different stages of intoxication, it was deemed desirably to study the power of contraction of the respiratory musculature itself during DFP intoxication. The respiratory muscle which is most appropriate for this purpose is the diaphragm. The most usual technique, apart from the X-ray one, is to open the pleural cavities. In those cases the animals must be given artificial respiration and spontaneous respiration can not be recorded. In order to get over this difficulty, the changes of the

Table 2.

Effect of DFP on the blood pressure and respiration of vagotomized rabbits. Effect of denervation of the sinus region on the 1st stage of intoxication.

Test No.	Weight of animal kg	Blood pressure and respiration before injection of DFP.				1st stage of the DFP-intoxication.									2nd stage of the DFP-intoxication according to:		
						Blood pressure and respiration before denervation of the sinus region. Increase or decrease per cent.					Blood pressure and respiration after denervation. Increase or decrease per cent.						
								Respiration				Respiration					
		Blood press. mm Hg	Resp. rate per min.	Volume of each respir. ml.	Ventilation ml. per min.	DFP mg	Blood press.	Rate	Volume	Ventil.	Blood press.	Rate	Volume	Ventil.	DFP mg.	Type I	Type II
12	2.8	120	30	23.3	700	0.8	— 8	+ 33	— 19	+ 5	— 8	+ 47	— 7	+ 36	1.3	+	
13	2.8	130	32	19.1	610	1.2	—31	+ 50	— 35	— 1	—42	+125	— 60	— 16	1.7	+	
14	2.7	106	44	20.8	919	1.1	+ 2	+ 23	— 32	— 17	+ 32	+ 5	— 32	— 29	1.9		+
15	2.9	—	27	20.0	540	1.0	—	+ 56	— 16	+ 30	—	+ 48	— 16	+ 36	1.8		+
16	2.8	120	47	25.0	1177	0.7	—47	+ 23	— 7	+ 15	—75	+ 23	— 47	— 34	0.7		+
17	2.4	100	52	18.3	955	0.8	—26	+ 54	— 36	— 2	—30	+ 54	— 50	— 23	0.8		+

Table 3.

Effect of DFP on the blood pressure and respiration of vagotomized and sinus-denervated rabbits.

Test No.	Weight of animal kg	Blood pressure and respiration before injection of DFP.				1st stage of the DFP-intoxication. Blood pressure and respiration. Increase or decrease per cent.					2nd stage of the DFP-intoxication according to:		
		Blood pressure mm Hg.	Resp. rate per min.	Volume of each resp. ml.	Ventilation ml. per min.	DFP mg	Blood pressure	Respiration			DFP mg	Type I	Type II
								Rate	Volume	Ventil.			
18	3.1	100	35	15.0	643	0.9	— 30	+ 20	— 22	— 24	1.3		+
19	2.8	80	23	38.3	883	0.4	± 0	+ 165	— 69	— 19	0.8	+	
20	2.3	90	42	16.7	701	0.6	± 0	+ 48	— 25	+ 11	0.9	+	
21	4.0	112	38	18.3	698	0.7	— 20	+ 47	— 45	— 20	0.7		+
22	3.3	110	36	20.0	721	0.8	— 2	+ 28	— 25	— 5	1.2		+
23	1.9	118	44	17.5	770	1.1	± 0	+ 41	— 26	+ 5	1.9	+	

Table 4.

Statistical analysis (t—test) of the values in table 1—3.

Variate	Mean deviation from the normal values per cent	Number of tests	Standard error of the mean	Degrees of freedom	t	P
A. Intact animals. Effect of DFP on: (table 1.)						
1. Blood pressure	+ 3.3	10	± 3.16	9	1.044	0.3—0.4
2. Rate of respiration	+ 46.1	11	± 12.25	10	3.763	0.001—0.01
3. Volume of each respiration	— 34.6	11	± 2.03	10	17.044	<0.001
4. Ventilation	— 4.8	11	± 7.81	10	0.615	0.5—0.6
B. a. Vagotomized animals (table 2)						
1. Blood pressure	— 22.0	5	± 8.40	4	2.619	0.1—0.2
2. Rate of respiration	+ 39.8	6	± 6.27	5	6.348	0.001—0.01
3. Volume of each respiration	— 24.2	6	± 4.86	5	4.979	0.001—0.01
4. Ventilation	+ 5.0	6	± 6.56	5	0.762	0.4—0.5
B. b. After denervation of the sinus region.						
1. Blood pressure	— 24.6	5	± 17.83	4	1.382	0.2—0.3
2. Rate of respiration	+ 50.3	6	± 16.70	5	3.012	0.02—0.05
3. Volume of each respiration	— 35.3	6	± 8.46	5	4.173	0.001—0.01
4. Ventilation	— 5.0	6	± 13.20	5	0.379	0.7—0.8
C. Vagotomized and sinus-denervated animals (table 3).						
1. Blood pressure	— 8.7	6	± 5.38	5	1.617	0.1—0.2
2. Rate of respiration	+ 58.2	6	± 21.80	5	2.670	0.05—0.1
3. Volume of each respiration	— 35.3	6	± 7.54	5	4.682	0.001—0.01
4. Ventilation	— 8.7	6	± 5.94	5	1.465	0.2—0.3
D. Differences between the effect of DFP on:						
1. Blood pressure of intact and vagotomized animals	+ 16.8	—	± 5.74	19	2.927	0.001—0.01
2. Rate of respiration of intact and sinus-denervated animals	— 8.2	—	± 21.30	21	0.385	0.7—0.8
3. Volumeof each respiration of intact and sinus-denervated animals	— 0.7	—	± 10.0	21	0.070	0.9
4. Ventilation of intact and sinus-denervated animals	+ 2.0	—	± 10.44	21	0.192	0.8—0.9

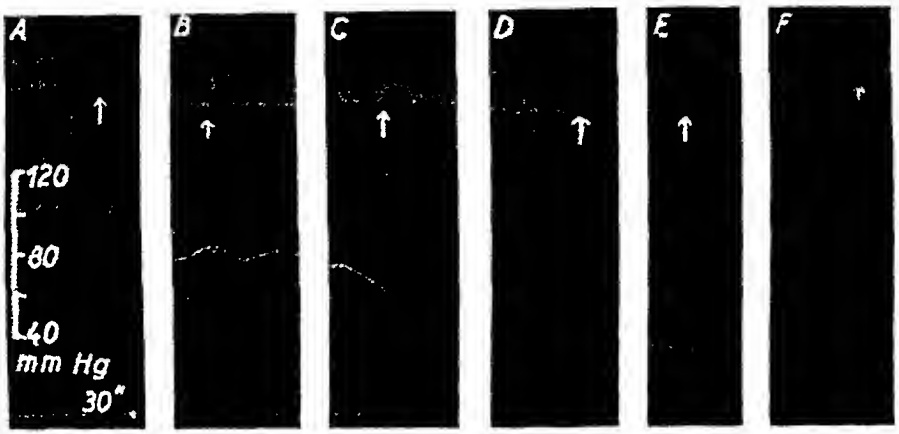

Fig. 2. Test 2. The effect of DFP on the response of the diaphragm to stimulation of the phrenic nerve. The arrows indicate stimulation.

A. normal respiration and blood pressure at 0^{00}. At 0^{01} 1.2 mg DFP.
B. at 0^{10}. At 0^{13} further 0.2 mg DFP. C. at 0^{14}.
D. at 0^{17}. At 0^{19} 20 mg atropine and artificial respiration.
E. at 2^{17}.
F. at 3^{37}.

respiration which follow on a tetanic contraction of the diaphragm were recorded instead. Fig. 2 A shows how, on stimulation of the phrenic nerve by an induction current of 50 per second, the respiration changes as a result of tetanic diaphragm contraction resulting in an increase of residual air. It should be pointed out that this method records an isotonic muscular contraction, but that the amplitudes is not directly proportional to the power of contraction.

The changes in respiration after DFP were ascertained, the power of contraction of the diaphragm being registered simultaneously on 9 rabbits, of which 6 were intact and 3 sinus-denervated. In all the cases a reduced contractive power was established (Fig. 2, B, C), but the degree of reduction varied. In the most conspicuous cases (tests 1 and 2), the contractive power of the diaphragm was altogether inhibited as early as during the first stage of the DFP intoxication, and respiration was maintained entirely by intercostal and auxiliary respiratory muscles. The second stage in those cases was of type 1. In five other cases (tests 2, 7, 8, 20, 21) the contractive power of the diaphragm was altogether inhibited simultaneously with the cessation of respiration (Fig. 2 D). In those cases the second stage was of type 1 in three cases. In the remaining two cases (tests 5, 18) the diaf

exhibited fairly good contractive power when respiration ceased. The second stage was of type 2 in these cases. Both these animals started to breathe again spontaneously after a few minutes of artificial respiration. It should be pointed out that in test 5, the animal did not exhibit increased respiration when oxygen lack appeared in spite of the sinus being intact. In test 18 the animal was sinus-denervated, and therefore it did not react with increased respiration either when oxygen lack appeared.

Blood Pressure.

During the first stage of DFP intoxication, blood pressure does not undergo any change in the case of intact rabbits. The pulse rate sometimes decreases however. With vagotomized rabbits, the blood pressure falls by 13.5 % on an average, which is statistically significant. The difference as between intact animals and vagotomized ones is also statistically significant (Table 4). During the second stage a fall in blood pressure only sets in after respiration has become definitely insufficient. After the cessation of respiration, too, the heart activity continues a few minutes, while the blood pressure falls. If artificial respiration is resorted to, the blood pressure rises a little, but will remain low even if artificial respiration is continued for a considerable time. If atropine is given, the rise in blood pressure is greater. After very large doses of DFP, the blood pressure falls quickly and the pulse rate declines. If artificial respiration is given, the heart activity does not cease, but the blood pressure continues to rise after the injection of adrenaline. On the whole, these results correspond with those described by MODELL et al. (l. c.).

Muscular Function.

In view of the paralyzing effect of DFP on the respiratory muscles it was considered to be of interest to study more closely its effect on the muscular function. The effect of DFP on the muscular function in the case of indirect stimulation has previously been investigated by MODELL et al. (l. c.) who observed a reduced response of the muscle. However, they interpreted the effect as a possible consequence of asphyxia. HUNT (1947) studied the effect of DFP on the neuromuscular transmission and states that, when given in small doses, DFP greatly increased the response of the

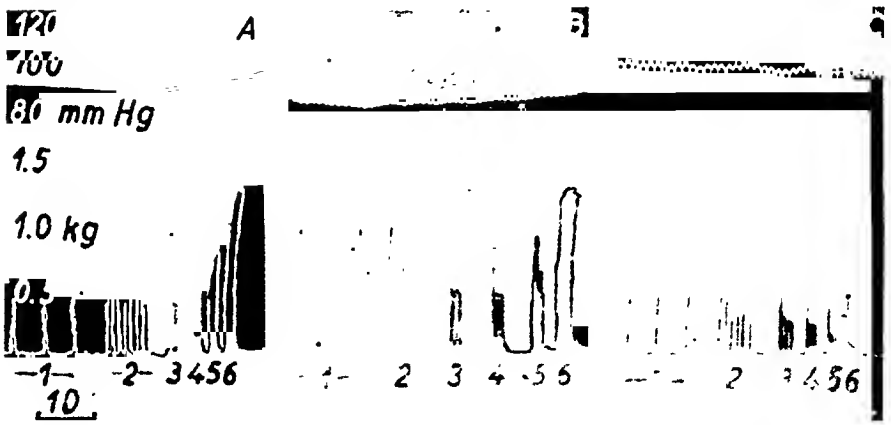

Fig. 3. Rabbit 2.4 kg. Above: Blood pressure. Below: Isometric recording of the right gastrocnemic muscle. Stimulation of the sciatic nerve by maximal break shocks of different frequencies. 1 = 1 in 5 seconds, 2 = 1 in 1 second. 3 = 4 in 1 second. 4 = 8 in 1 second. 5 = 16 in 1 second, 6 = 50 in 1 second. Artificial respiration and 20 mg atropine was given before DFP.

A. normal response at 0^{00}. At 0^{02} 3.3 mg DFP.
B. at 0^{10}. At 0^{13} further 5.5 mg DFP.
C. at 0^{20}.

gastrocnemius of the cat to maximal single nerve shocks, whereas with larger doses an inhibition is to be observed.

In order to render possible a study of the effect of large doses of DFP on the muscular function, the animals were given artificial respiration and 10 mg/kg atropine i. v. before DFP was administered. Animals treated in such a manner do not exhibit any fall of blood pressure, even after the administration of 20 times the normal lethal dose. It was not found that atropine had any influence on the effect of DFP on the muscular function. After 1—2 mg/kg of DFP i. v., stimulation of the nerve with maximal break shocks of low frequency (1 in 5—10 seconds), greatly increases the response of the muscle (Fig. 3 B). If the frequency of stimulation is increased, the responses decrease. Only the first twitch in each series is greatly increased (Fig. 3 B). At this stage the tetanic contraction remains unchanged.

If the dose of DFP is increased to 4—6 mg/kg the response of the muscle to single nerve shocks decreases with an increase of the frequency of stimulation. The tetanic contraction is also distinctly reduced (Fig. 3 C). It becomes more like a single twitch, and both the power of contraction and the ability to retain the contraction at its original strength decrease. If the nerve is stimulated continuously with a successive increase of the frequency of shocks,

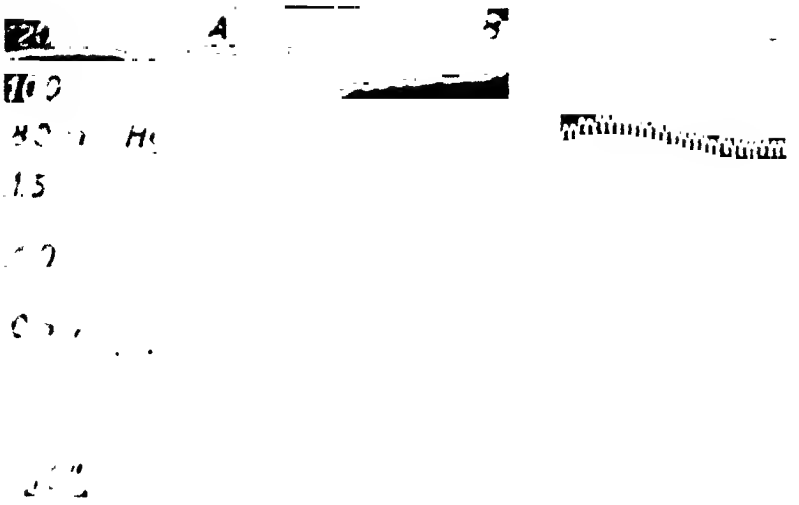

Fig. 4. Rabbit 2.2 kg. Isometric recording of the gastrocnemic muscle. Direct stimulation of the muscle by maximal break shocks. Artificial respiration and 20 mg atropine was given before DFP.

A. normal single twitch and tetanic contraction at 0^{00}. At 0^{01} 1.5 mg DFP.
B. at 0^{15}. At 0^{18} 3.5 mg d-tubocurarine chloride i. v. C. at 0^{35}.

the power of contraction of a normal muscle will increase when summation of the contractions begins but a muscle treated with DFP behaves contrariwise, the power of contraction decreasing with an increase of frequency. No complete depression of the response to indirect stimulation is obtained, even after 10 mg/kg of DFP.

On direct stimulation of the muscle the responses to single maximal break shocks are increased to the same degree as with indirect stimulation (Fig. 4 B). If however the nerves and the neuromuscular transmission are blocked by curarine the increase is inhibited (Fig. 4 C). The tetanic contraction is well maintained during the whole of the intoxication. Even after 10 mg/kg DFP both the response to single shocks and the tetanic contraction are now unaffected by direct stimulation.

In some tests the response of the tibialis muscle to indirect stimulation was investigated simultaneously with that of the diaphragm. In these case it turned out that the power of contraction of the diaphragm was nearly completely depressed at the stage when the response of the tibialis anticus muscle to single nerve shocks was increased without any reduction of the tetanic contraction. Therefore it seems as if the diaphragm were specially sensitive to DFP. On the other hand, the effect of DFP on the

diaphragm disappears more rapidly. In one test no improvement in the response of the tibialis muscle to indirect stimulation could be noticed even 10 hours after DFP had been given, although the animal could breathe spontaneously already after 5 hours. Large doses of atropine had no effect on the muscular function after DFP, whereas magnesium sulphate, which has been proposed by Mc NAMURA, KOELLE and GILMAN (l. c.) for the treatment of DFP intoxication, further reduces the muscular function.

Treatment of DFP Intoxication.

It has already been mentioned that an animal intoxicated by DFP can be kept alive by artificial respiration. If atropine is given simulataneously, the blood pressure rises, and the animal may be in fairly good condition even after large doses of DFP. It turned out that if the animal was kept alive in that way, the spontaneous respiration was re-established. In cases where the contracting power of the diaphragm was good, spontaneous respiration reappeared after a few minutes of artificial respiration, whereas when the contractive power of the diaphragm disappeared, spontaneous respiration only returned after 1—5 hours of artificial respiration, simultaneously with the return of the contracting power of the diaphragm (Fig. 2 F). At first, however, spontaneous respiration was insufficient and ceased altogether after 5—10 minutes, whilst the blood pressure was falling. If artificial respiration was again resumed the respiration gradually became sufficient. The case which was followed longest showed no fall of blood pressure nor decreased respiration for an hour. In those cases where respiration had already ceased and the blood pressure had gone down to 0, adrenaline and heart massage had sometimes to be given in order to bring about a rise of blood pressure. In order to secure the maximum effect of atropine, large doses (up to 10 mg/kg) had to be given. It should be pointed out, however, that rabbits are remarkably insensitive to atropine.

Discussion.

Cause of Death.

As appears from the foregoing, DFP chiefly affects the respiration. A definite insufficiency of respiration can be established even before the blood pressure begins to fall. Heart activity also con-

tinues after the cessation of respiration and ceases only owing to asphyxia, which is proved by the facts that artificial respiration causes a rise of blood pressure and that heart activity continues as long as artificial respiration is maintained. Thus the proximate cause of death is a paralysis of respiration, as has been pointed out by most investigators (COON, l. c., MODELL, KROP, HITCHCOCK and RIKER, l. c., MC NAMURA, KOELLE, GILMAN, l. c., SCHOLZ, l. c.). This holds when the doses of DFP are only moderatly large. With very large doses both respiration and heart activity are rapidly inhibited, but even in this case paralysis of respiration is probably the direct cause of death, when the heart activity does not cease altogether.

Effect on Respiration.

The first stage of DFP intoxication is characterized by a decrease of the volume of respiration and an increase of the frequency of respiration. During this stage a decrease in the contractive power of the diaphragm occurs, and therefore the primary factor is probably the decreased volume of each respiration, whilst the increase in the frequency is of a compensatory nature to keep up ventilation. In 23 tests the total ventilation decreased by only 3.5 % on an average. As the volume of respiration decreases, the dead space becomes relatively larger, the alveolar ventilation having decreased more than is indicated by the values for total ventilation. Therefore it is probable that there is a slight asphyxia during the first stage of DFP intoxication, and thus no stimulated respiration as suggested by MODELL et al. (l. c.).

The second stage of DFP intoxication is characterized by a progressive insufficiency of respiration. This insufficiency is due to a distinctly decreased contractive power of the muscles of respiration. In the two cases where the diaphragm lost its contractive power at a very early stage, it is possible that cessation of respiration was due entirely to a peripheral paralysis. In the two cases where cessation of respiration set in, whilst the diaphragm still showed good contractive power, it must be assumed that this was due to a central paralysis. In the five cases where the diaphragm lost its contractive power simultaneously with the cessation of respiration, it is more difficult to decide whether there was a peripheral or a central paralysis or both. Those cases where the auxiliary muscles of respiration came into play (Type I) showed the existence of a functioning respiratory centre, wherefore the

paralysis of respiration may be interpreted as a peripheral one in those cases too. In the cases where the auxiliary muscles of respiration did not come into play (Type 2), this may have been due both to a central paralysis and to a loss of contractive power in these muscles. That the first alternative may be the right one is indicated by the fact that, after a couple of minutes of artificial respiration, the auxiliary muscles of respiration came into play, and that Type 2 therefore may pass over into Type 1. In the cases when the cessation of respiration was of Type 1, a peripheral paralysis may have been the cause, whereas in the cases where it was of Type 2 a central paralysis was the cause.

The next question is whether the paralysis of the respiratory centre was a direct effect of DFP, as assumed by MODELL et al. (l. c.) This is not likely and for the following reasons. By means of artificial respiration for 3—5 minutes and by the administration of atropine to increase the blood pressure, it was possible in five cases out of six to reverse the central paralysis, *i. e.* transform Type 2 into Type 1. Therefore the central paralysis of respiration may be a result of hypoxia. By artificial respiration this hypoxia is reversed, and the centre of respiration again begins to function. This might also explain why sinus-denervated animals exhibit an early paralysis of respiration of a central nature more than animals with intact carotid body. It is well known that in the case of sinus-denervated animals respiration is apt to fail when there is a lack of oxygen, the cause being hypoxia of the centre of respiration (HEYMANS, BOUCKAERT and DAUTREBANDE l. c., v. EULER and LILJESTRAND l. c.). The tests (3, 18) in which cessation of respiration occurred in spite of satisfactory contractive power of the diaphragm also indicate that the central paralysis of respiration is caused by hypoxia. In both these cases the compensatory reaction when there was lack of oxygen was bad.

The effect of DFP on respiration may be explained as follows. DFP decreases the volume of each respiration by reducing the contractive power of the musculature of respiration. To compensate this decrease the frequency increases, so that the total ventilation is not changed, whereas the alveolar ventilation decreases and a slight asphyxia sets in (= first stage). When the contractive power of the musculature is further decreased, respiration becomes still more insufficient. In some cases, especially when the compensatory reaction to lack of oxygen (sinus-denervated animals) is absent, or the blood pressure falls in an early

stage of intoxication the centre of respiration quickly becomes paralyzed owing to hypoxia, without the auxiliary muscles of respiration having time to come into play. In other cases, with a more resistant centre of respiration, where the compensation for lack of oxygen is better, and the fall of blood pressure comes later the auxiliary muscles of respiration come into play. In some cases ventilation thus becomes sufficient for some time, but with a further weakening of the muscular function, sooner or later the stage is reached when the ventilation becomes so insufficient that the respiratory centre is paralyzed by hypoxia.

Blood pressure.

The fall of blood pressure in DFP intoxication may be attributed chiefly to the parasympathomimetic effect of DFP on heart and vessels. Whether DFP also has a directly paralyzing effect on the vasomotor centre in the medulla, as had been assumed by MODELL et al. (l. c.), cannot be determined definitely by the method of procedure employed. This possible paralysis will not be of any great importance, because after the inhibition of the peripheral effect of DFP by means of atropine, the blood pressure almost attains its original value. It is, however, probable that the vasomotor centre is affected by asphyxia as the blood pressure rises somewhat after artificial respiration. To what extent the fall in blood pressure is due to an inhibition of the heart function or to a peripheral vasodilation has not been finitely determined. The greatly reduced pulse rate is, however, an indication that the chief importance must be attached to an inhibition of the heart function. No plausible explanation of why, at an early stage, DFP reduces the blood pressure on vagotomized but not on intact animals can be given. The one which most readily suggests itself is that the increase in blood pressure caused by vagotomy will in the first place be inhibited by DFP. Such an assumption is however negatived by the fact that in the case of both intact animals and vagotomized ones the initial blood pressure was the same on an average.

Muscular Function.

The effect of DFP on the muscular function may be due either to the nerves or to the neuromuscular transmission or to the muscles being directly affected. Of these alternatives, a direct effect on the muscles may be excluded, because, after nerves and

neuromuscular transmission have been blocked by curarine, DFP has no effect on muscular contractions after direct stimulation. The nerves, too, may be considered to be a less likely point of attack, for BULLOCK, GRUNDFEST, NACHMANSOHN and ROTHENBERG (1946, 1947 a. b. c.) and CRETCITELLI, KOELLE and GILMAN (1946) have shown that only at a concentration of 2—4 g/kg has DFP any effect on the action potentials of the nerves. This concentration is almost 1000 times as great as the one employed in the investigation described above. Therefore the neuromuscular transmission will be the most likely point of attack, as has also been assumed by MODELL et al. (l. c.) and HUNT (l. c.). So far the result obtained will be pretty certain. The further explanation is, however, of a more hypothetical nature.

According to the teory of humoral transmission, on the stimulation of the motor nerves, acetylcholine is released, and this gives rise to a muscular contraction. This acetylcholin is quickly destroyed by the cholinesterase present at the end-plates of the muscles. If, owing to DFP, the activity of the cholinesterase is reduced, the acetylcholine released has a prolonged effect and gives rise to a short tctanic contraction. As assumed by HUNT (l. c.), the potentiated responses of the muscle to maximal single nerve shocks may be short tetanic contractions. Physostigmine causes a similar potentiation, and BROWN, DALE and FELDBERG (1936) have shown that these are short tetanic contractions. If the activity of cholinesterase is still more reduced, acetylcholine will be assembled at the end-plates, especially if the frequency of stimulation is rapid, and this results in the blocking of neuromuscular transmission. For SIMONART (1936) and BROWN, DALE and FELDBERG (l. c.) have shown that high concentrations of acetylcholine block the neuromuscular transmission.

Treatment.

By means of artificial respiration and atropine, it seems to be possible to keep a badly intoxicated animal alive, until the acute symptoms of intoxication are over. Whether the life of the animal may be definitely saved thereby or whether it succumbs later in the condition ensuing upon DFP-intoxication remains to be investigated. The treatment described above may possess the advantage over the one suggested by MC NAMURA, KOELLE and GILMAN (l. c.) — viz. the employment of atropine — magnesium sulphate — that it can be initiated at a far advanced stage of in-

toxication. The therapeutic effect of atropine reported by MODELL and KROP (1946) may be explained by the fact that, owing to the inhibition of the fall of blood pressure after DFP the animals tolerate a greater reduction of the ventilation before the respiratory centre is paralyzed by hypoxia.

Summary.

The effect of DFP on the rabbit has been investigated as regards respiration, blood pressure and muscular function.

1. The effect on respiration takes place in two stages. During the first stage, the volume of each respiration diminishes, the frequency of respiration increases, whilst the total ventilation remains unaltered. During the second stage, respiration gradually becomes insufficient and ends in a cessation.

2. Denervation of the sinus region does not change the first stage. An earlier cessation of respiration occurs more often than in the case of intact animals.

3. Pari passu with the diminution of the volume of respiration, the contracting power of the diaphragm is reduced on indirect stimulation.

4. The primary effect on respiration is therefore a reduction in the volume of respiration, consequent upon a decrease in the contractive power of the respiratory musculature. To compensate this, the frequency increases and the total ventilation becomes constant. The alveolar ventilation diminishes, however, and a slight asphyxia follows. Owing to a further reduction of the contractive power of the respiratory musculature, ventilation is reduced and the animal becomes more asphyctic. As a result of the hypoxia the centre of respiration becomes paralyzed. In some cases this paralysis sets in whilst the respiratory muscles still retain a certain contractive power, in other cases the respiratory centre is paralysed only when the contractive power has almost entirely disappeared.

5. During the first stage the blood pressure undergoes no change in the case of intact rabbits. In the later stages, the blood pressure goes down and the pulse rate decreases continuously.

6. By the administration of atropine and by artificial respiration after the cessation of respiration, intoxicated animals may be kept alive.

7. Muscular function was examined on the gastrocnemic and the tibialis anticus muscles in situ. The response of the muscle to maximal single nerve shocks is increased after 1—2 mg DFP/kg i. v., with low frequency of stimulation. With high frequency stimulation, the response of the muscle is unchanged. If the dose of DFP is increased to 4—10 mg/kg, the response to single nerve shocks decreased. The response of the muscle decreases as the frequency of stimulation increases. The tetanic contraction is greatly reduced, both the power of contraction and the ability to retain the contraction at its original stage decreasing. On direct stimulation of the curarized muscle no effect on the muscular function can be determined, even after 10 mg/kg of DFP.

The action of DFP is localized in the neuromuscular transmission.

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Effects of Adrenaline and Ergotamine on the Oxygen Consumption.

By

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From numerous investigations it is already known that adrenaline increases the oxygen consumption both in the case of animals in toto and in isolated tissues. Since DALE (1906) discovered the adreno- and sympaticolytical effect of the ergot alkaloids it has proved possible to inhibit more and more of the stimulating and inhibiting effects of adrenaline with alkaloids contained in ergot. A number of authors have investigated whether the increased oxygen consumption after adrenaline is inhibited by ergot alkaloids.

Thus v. EULER (1929) proved by means of Thunberg's methylene blue method that ergotamine in a concentration of 10^{-12}—10^{-6} completely inhibited the increase in metabolism which adrenaline in a concentration of 10^{-12} gave rise to in minced frog muscle. v. EULER also found that, in the case of perfusion of isolated hind leg of the dog and analysis of the perfused blood, ergotamine inhibited the increase of oxygen consumption after adrenaline.

CAPO (1930) investigated on man whether 0.5 mg of ergotamine inhibited the increase in oxygen consumption after 1 mg of adrenaline. The drugs were administered subcutaneously. In four instances ergotamine and adrenaline were given simultaneously. In one case adrenaline was administered 20 min. after ergotamine. In no case was an inhibition of the adrenaline effect on oxygen consumption obtained.

YOUMANS, TRAUBE and BUVINGER (1933) also examined the effect on man of 0.5 mg of ergotamine on the increase in oxygen consumption after both 1.0 mg and 0.5 mg of adrenaline. Ergotamine was given subcutaneously both 10—15 minutes before adrenaline and immediately afterwards. Three tests were made with each group. The authors consisered that they could prove that a partial inhibition could be traced if ergotamine was given after 1 mg of adrenaline. The experimental foundation for this assumption, however, is hardly convincing. A study of the results indicates rather that no inhibition occurred, but that the differences found were a matter of chance.

ORESTANO (1933) states that in the rat 1.1 mg of ergotamine per kg of body weight partly inhibited the increase in oxygen consumption after adrenaline. In order to obtain complete inhibition, the dose had to be increased to 10—20 mg/kg. The author states, however, that 3 mg of ergotamine per kg of body weight distinctly reduces the metabolism and that 10—20 mg of ergotamine per kg of body weight reduces the metabolism by 30 % or just as much as the adrenaline dose (not stated) increases it. The inhibition is most clearly shown if ergotamine and adrenaline are injected simultaneously. Therefore it is possible that these results are not due to a specific inhibition of adrenaline by ergotamine, but that the reducing effect of ergotamine on the oxygen consumption and the increasing effect of adrenaline compensate each other, so that oxygen consumption does not undergo any change. As the author does not indicate the results of the primary tests nor the number of the tests, an appraisement of his work is rendered more difficult.

HARANGOZO-OROSZY and ISSEKUTZ (1942) used rats anesthetized with urethane to investigate whether 5—10 mg of ergotamine per kg of body weight inhibited the effect of 0.37—0.57 mg of adrenaline per kg of body weight. Both drugs were administered intraperitoneally. According to these authors the effect of adrenaline on the oxygen consumption is biphasic, the oxygen consumption first decreasing during 10—20 minutes, and then increasing. Whilst the decrease in oxygen consumption was inhibited by ergotamine, the following increase was not affected. It should be mentioned that, after the ergotamine injection, the oxygen consumption went down by 8 to 25 % below the normal value and that the following increase after adrenaline exceeded the normal value but little or not at all. Although the authors

did not check whether the decrease after ergotamine was maintained unchanged during the whole test, they base their calculation of the increase after adrenaline upon that figure. Therefore, the objection may be raised against these tests that the changes in the oxygen consumption can be attributed to ergotamine.

In brief, it may be said that in the case of isolated organs and tissues, ergotamine has proved to inhibit the increase of metabolism after adrenaline. In the case of animals and man in toto the results are partly negative and partly so doubtful that no definite conclusions can be drawn.

The tests described below were carried out with ergotamine. In planning the tests the following considerations were borne in mind.

As previously mentioned, large doses of ergotamine reduce the metabolism, as has been confirmed by tests made by MARINE, DEUTCH and CIPRA (1927). Therefore it was considered appropriate to give small doses of ergotamine, which are adrenolytic but do not affect the metabolism. The adrenolytic limit concentration of ergotamine for mammals is 16—33 γ per kg of body weight (RAYMOND-HAMET 1926). Also with adrenaline it proved desirable to work with small doses, in order to ascertain the specific effect on metabolism which is clear even with very low degrees of concentration, and to avoid toxic effects which might have a secondary effect on metabolism. Nor is the adrenolytic effect of ergotamine absolute. If the concentration of adrenaline is increased, the concentration of ergotamine must also be increased if the inhibition is to be complete (GADDUM 1926). If a small dose of ergotamine is given, the dose of adrenaline must also be small, for a possible inhibition to be proved. In the case of man 5—10 γ of adrenaline per kg of body weight increases the oxygen consumption by 20 to 30 % (BOOTHBY and SANDIFORD 1922, EULER and LILJESTRAND 1927), and for dog 20 γ per kg of body weight increases the oxygen consumption just as much (MOERLOOSE 1942). 20 γ per kg of body weight of both adrenaline and ergotamine was considered to be a suitable dose. Further it was considered advisable to give the adrenaline only after the ergotamine had been completely resorbed. MOIR (1932) found that the pregnant human uterus in situ contracted 4—10 minutes after an intravenous injection of ergotamine, whereas with an intramuscular injection, the uterus only contracted 15—45 minutes after the injection. Therefore it was considered advisable to inject the adrenaline only about 30 minutes after the ergotamine had been given intramuscularly.

The first tests were carried out on cats anesthetized with chloralose, but it turned out that only with a dose of 1000 γ of adrenaline per kg of body weight could a definite increase of the oxygen consumption be proved. Therefore it seemed as if the anesthesia inhibited the increase of oxygen consumption after adrenaline, which has also been suggested by KLEIN and WEISS (1928) and MOERLOOSE (*l. c.*). The further trials were consequently carried out on non-anesthetized animals, which had

the additional advantage that several tests could be carried out on one and the same animal.

Method.

The oxygen consumption was determined by means of an apparatus described by KROGH and LINDBERG (1931). The apparatus is a closed system with a container for the animal. A pump drives the air in the container through a flask of soda lime, which absorbs the carbon dioxide, and a flask of "blue gel" which absorbs the water vapor. The system is connected up with a spirometer of the Hutchinson type, which records the volume changes in the system. In the first tests, a bellows pump in the shape of a rubber bladder was employed, which, by compression and expansion, drove the air in the desired direction through a valve. As the rubber bladder proved to have too short a lifetime it was replaced by a rotary pump. The container, which was closed with a lock of liquid paraffin, was submerged in a water-bath at 26° C, as were also the absorption vessels. The containers were of two sizes: one with a capacity of 2 litres for guinea pigs, and one of 10 litres for cats. For the smaller container was used a spirometer of 500 cc capacity, and for the larger one a spirometer of 2000 cc capacity was used. The pump was so regulated that, when the smaller container was used, the speed of the air current was about 2 litres per minute, whereas when the larger container was used it was 8—10 litres per minute. Before the first test every day, the apparatus was allowed to idle for 20—30 minutes in order to check its tightness and to level out any differences in temperature. After the animals had been placed in the container, the system was filled with oxygen, the recording starting 5 minutes afterwards, the kymograph being moved forwards a few millimeters. In the first test every period of 30 minutes was marked by hand by advancing the kymograph; in the later tests the marking was effected by means of a clock, which via an electromagnet caused the kymograph to move forwards exactly every fifth minute. At the beginning of the test, the analysis of the gas in the system showed a percentage of 90—95 of oxygen and 0.03—0.05 of carbon dioxide. After 60 minutes the percentage of oxygen had gone down to 85—90 whereas the percentage of carbon dioxide was unchanged. The spirometer being filled with liquid paraffin, there were no free water surfaces in the system. Hygroscopic determinations during the tests showed a relative humidity of 0 %, so, that when correcting the quantity of oxygen consumed to 0° C and 760 mm Hg, the gas was considered to be dry. The vapor pressure of the liquid paraffin was ignored, as it only amounted to 1—2 mm Hg and underwent no change under the experimental conditions.

Guinea pigs and cats were used as test animals. Guinea pigs being quiter and easier to handle than cats, most of the investigations were carried out on the former. The animals were given no food for 16—24 hours previous to the tests.

Results.

In the first test series, comprising 7 guinea pigs, 5 tests were made on each animal. In each test, the normal oxygen consumption during 60 minutes was first determined. In one test on every animal, the injection effect was investigated. 1 cc of 0.9 % NaCl was injected subcutaneously in the back, and the oxygen consumption during 15—45 minutes after the injection was recorded (control tests). In two tests the effect of adrenaline was investigated. 10 γ per animal (20 γ per kg body weight) of adrenaline hydrochloride (Leo) in 1 cc of 0.9 % NaCl was injected subcutaneously in the back, the oxygen consumption then being recorded 15—105 minutes after the injection (adrenaline tests). In two tests the animals were injected intramuscularly in one hind leg with 10 γ ergotamine tartrate (Gynergen, Sandoz) in 1 cc of 0.9 % NaCl, corresponding to about 20 γ of ergotamine per kg of body weight. After the injection, the oxygen consumption was recorded during 10—40 minutes. Then 10 γ of adrenaline was given subcutaneously and the oxygen consumption was recorded for 15—105 minutes (ergotamine-adrenaline tests). The results are assembled in Table 1. The oxygen consumption is given in cc/min. The percentage of deviation from the normal values is also shown.

The normal values vary a great deal from day to day, which conforms with the findings of other investigators (KROGH and LINDBERG, l. c.). In this investigation, however, this variation is of secondary importance. As appears from Table 1 the effects of the drugs also vary considerably. A statistical treatment of the material was therefore deemed necessary. For this treatment, the percentages of deviations from normal values have been employed, and the statistical probability of the differences has been calculated according to the t-analysis (FISCHER 1936). On Table 2 the statistical analysis is summarized. P-values less than 0.05 have been considered worth discussing and P-values less than 0.01 have been considered significant.

As appears from Table 2, the increase of oxygen consumption in relation to the normal values is statistically significant both in the adrenaline and the ergotamine-adrenaline tests. No definite effect of ergotamine or the control injection during the first 30 minutes after the injection can be proved. The difference between the adrenaline and the ergotamine-adrenaline tests is also significant, wherefore it is very likely that ergotamine inhibited the

Table

The effect of adrenaline, ergotamine-adrenaline, and control tests,

Guinea-pig No.	Weight gm	Adrenaline γ/kg	Ergotamine-tartrate γ/kg	Control tests. Injection of 1 ml 0.9% NaCl subcutaneously		
				Oxygen consumption cc/min.		Increase or decrease per cent
				60—0 min. before injection	5—35 min. after injection	
I.	670	30	15	8.2	8.8	+ 7
				—	—	—
II.	550	18	18	9.1	10.2	+ 12
				—	—	—
III.	590	17	17	8.0	8.1	+ 2
				—	—	—
IV.	470	21	21	6.4	6.9	+ 7
				—	—	—
V.	550	18	18	7.1	7.0	— 1
				—	—	—
VI.	450	22	22	7.8	8.2	+ 4
				—	—	—
VII.	440	23	23	7.7	7.1	— 8
				—	—	—

increase of oxygen consumption after adrenaline. However, this cannot be said to be fully proved since the difference may also be due to a decreasc in the oxygen consumption owing to ergotamine. Certainly no traceable change in the oxygen consumption takes place during the first 30 minutes after injection of ergotamine, but as ergotamine is absorbed slowly, a decrease may take place later. The increase of the oxygen consumption in the ergotamine-adrenaline tests might be due to the inhibition by adrenaline not having been complete. If the difference as between ergotamine-adrenaline and control tests is calculated, this difference is not significant; thc increase in oxygen consumption after ergotamine-adrenaline may also be due to the injections. It should, however, be pointed out that, whilst the oxygen consumption in the ergotamine-adrenaline tests was determined during 90 minutes, that in the control tests was only determined during 30 minutes after thc injection. Therefore it may be assumed

1.

on the oxygen consumption of guinea pigs. The first test series.

Adrenaline tests			Ergotamine-adrenaline tests				
Oxygen consumption cc/min.		Increase or decrease per cent	Oxygen consumption cc/min.		Increase or decrease per cent	Oxygen consumption cc/min. 5—95 min. after injection of adrenaline	Increase or decrease per cent
60—0 min. before injection	5—95 min. after injection		60—0 min. before injection	5—35 min. after injection of ergotamine			
8.7	8.9	+ 2	10.7	9.3	— 13	10.6	— 1
10.2	12.3	+ 21	10.1	9.0	— 11	10.1	0
7.3	10.0	+ 37	7.1	6.5	— 9	7.4	+ 4
7.4	7.8	+ 5	8.1	8.0	— 1	9.3	+ 15
8.8	10.0	+ 14	7.9	7.7	— 3	7.3	— 8
8.5	9.0	+ 6	8.6	10.2	+ 19	9.2	+ 7
8.0	8.5	+ 6	6.2	6.8	+ 9	6.6	+ 7
7.9	8.3	+ 5	6.6	6.0	— 9	6.7	+ 2
6.9	9.2	+ 33	7.7	7.0	— 8	8.1	+ 5
7.4	8.0	+ 8	7.4	7.4	0	7.6	+ 3
7.1	7.7	+ 9	7.7	8.2	+ 6	8.3	+ 8
6.2	6.6	+ 7	5.8	5.8	0	6.1	+ 5
7.4	9.1	+ 23	8.4	8.9	+ 6	8.6	+ 2
8.5	10.1	+ 26	8.4	8.7	+ 3	9.4	+ 11

that the control tests exaggerate the injection effect in relation to the ergotamine-adrenaline tests, and the difference found is a minimum value.

As in the first test series insufficient account was taken of the effect of ergotamine on the oxygen consumption or of the injection effect, a fresh series of tests was carried out on 5 animals, 10 tests being made on each animal. In order to eliminate the effect of the injection in relation to the effect of the various drugs upon oxygen consumption, the animals got a subcutaneous injection of 1 cc of 0.9 % NaCl 5 minutes previous to the commencement of the recording of the oxygen consumption (control injection). The oxygen consumption was then recorded in periods of 5 minutes during 60 minutes. The different drugs were then injected, and the oxygen consumption was determined in all the cases during 5—95 minutes after the last injection. The doses of adrenaline and ergotamine are given below in γ/kg of body weight.

Table 2.

Statistical analysis of the values of table 1.

Variate	Mean deviation from the normal values per cent	Number of tests	Standard error of the mean	Degrees of freedom	t	P
Control tests	+ 3.3	7	± 2.45	6	1.348	0.3—0.4
Adrenaline tests .	+ 14.4	14	± 3.05	13	4.721	< 0.001
Ergotamine tests 5—35 min. after injection	— 0.8	14	± 2.39	13	0.335	0.7—0.8
Ergotamine adrenaline tests	+ 4.3	14	± 1.45	13	2.965	0.01—0.02
Difference between Adrenaline tests and Control tests	+ 11.1	—	± 3.91	18	2.839	0.01—0.02
Difference between Ergotamine tests and Control tests....	— 4.1	—	± 3.38	18	1.198	0.2—0.3
Difference between Ergotamine-adrenaline and Control tests	+ 1.0	—	± 2.85	18	0.351	0.7—0.8
Difference between Adrenaline tests and Ergotamine-adrenaline tests	+ 10.1	—	± 3.38	26	2.989	0.001—0.01

Each animal was subjected to: 2 tests with 20 γ of adrenaline subcutaneously (adrenaline tests), 2 tests with 20 γ of ergotamine i. m. in one hind leg (ergotamine tests), 3 tests in which first 20 γ of ergotamine was given i. m., and after 15 minutes 20 γ of adrenaline (ergotamine-adrenaline tests), 3 tests when 20 γ of adrenaline was first given subcutaneously and after 10 minutes 20 γ of ergotamine i. m. (adrenaline-ergotamine tests). The last group of tests was made in order to discover whether a manifest increase of oxygen consumption after adrenaline is also inhibited by ergotamine.

Fig. 1 shows the effect on oxygen consumption of the control injections. The average values of the first two 5-minute periods on the five animals have been correlated with the number of injections given to each animal. Between the control injections the animals were given 1—2 injections of adrenaline or ergotamine.

In Table 3 is shown the statistical analysis of the material. The oxygen consumption is moderately increased 5—10 minutes after the injection, which increase is significant. No change in the oxygen consumption can be traced 10—15 minutes after the injection or during the immediately following periods. Therefore a constant

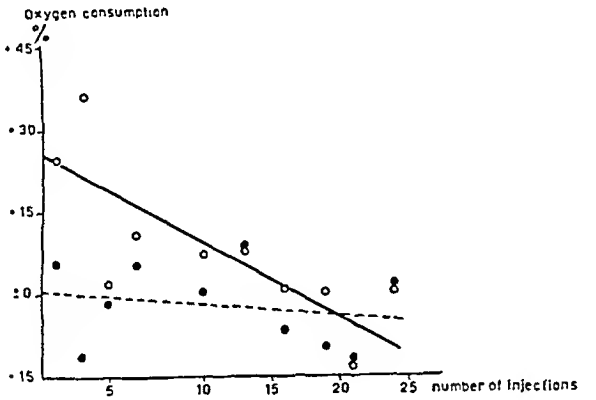

Fig. 1. The effect of the control injections on the oxygen consumption correlated with the total number of injections given to the animal. Abscissa: The number of injections. Ordinate: Percentage of change in the oxygen consumption. Each point represents the average of 5 tests. 0—0: The first period, 5—10 minutes after injection, and the line of regression. ● ···●: The second period, 10—15 minutes after injection, and the line of regression.

oxygen consumption may be probably assumed already 10 minutes after the injection. The increase in oxygen consumption has also been correlated with the number of injections, and there exists a significant correlation showing that the increase in the oxygen consumption decreases with the number of injections. Thus the animals may become accustomed to the injections.

The results of the drug tests are assembled in Table 4. The normal values have been obtained as follows: — To the oxygen consumption during the first 30 minutes has been added twice the oxygen consumption for the last 30 minutes, the value obtained being divided by 90. This correction has been deemed necessary in order that the injection effect may not play a relatively greater rôle in the control tests, where the oxygen consumption was measured during 60 minutes after the injection, than in the drug tests, where the oxygen consumption was measured during 90 minutes. In the first tests, in which the injection effect is great, the normal value without this correction would be to high. In

Table

Statistical analysis of the effect of the

Variate	t-analysis		
	Mean deviation from the normal values per cent	Standard error of the mean	Degrees of freedom
First period 5—10 min. after control injection	+ 7.5	± 2.90	49
Second period 10—15 min. after control injection	—2.4	± 2.33	49

the later tests, in which the injection effect is low, it is of no importance.

In Fig. 2 the relation between the increase of the oxygen consumption and the duration of the effect of the various drugs is

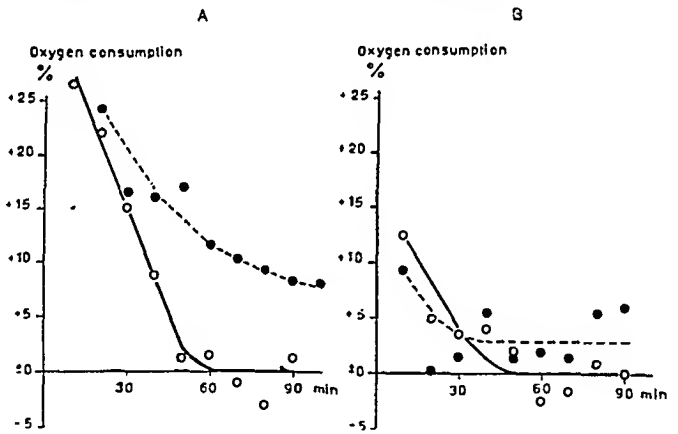

Fig. 2. Time-action curves for adrenaline, ergotamine, and a combination of the two. Abscissa: Time in minutes after the injection of adrenaline and ergotamine respectively. Ordinate: Percentage of change in the oxygen consumption. A. Tests with adrenaline o—o; tests with adrenaline-ergotamine ● ●. B. Tests with ergotamine-adrenaline o—o; tests with ergotamine ● ●.

shown. The initial increase in the ergotamine and the ergotamine-adrenaline tests may be an injection effect. From Fig. 2 it emerges that the effect of adrenaline disappeared after 60 minutes. The adrenaline-ergotamine curve probably reflects the actual facts better, as it corresponds more closely to a curve based upon a larger number of tests (LUNDHOLM, unpublished). The statistical analysis of the material is collected in Table 5. The increase

3.

control injections on the oxygen consumption.

			Correlation			
t	P	Degrees of freedom	b	r	t	P
2.598	0.01—0.02	48	—1.54 %	—0.571	4.818	< 0.001
1.013	0.3—0.4	48	—0.24 %	—0.109	0.754	0.4—0.5

in the adrenaline and adrenaline-ergotamine tests is significant. No certain effect of ergotamine or ergotamine-adren-aline can be proved.

The results of the second test series correspond with those of the first series. In order to use the material in the best possible way, all the adrenaline and the ergotamine-adrenaline tests have been statistically worked up, the results being given in Table 6. This procedure has been considered justifiable as the two series only differ in respect of the control injections. As pointed out above, the effect of the control injection had already disappeared in 10 minutes. In the first series of tests, the recording of the oxygen consumption was only begun 15 minutes after the injection, so that the injection effect can be disregarded in this case. Whether ergotamine inhibits the increasing effect of adrenaline on the oxygen consumption can now be decided, with great certainty. If there is no inhibition, the effect on oxygen consumption in the ergotamine-adrenaline tests should be the same as the sum of the average effects in the ergotamine and the adrenaline tests. From Table 6, however, it appears that there is a difference between the effects in these tests, and the probability that this is due to chance is only 0.001—0.01. Therefore there is every probability that ergotamine inhibits the effect of adrenaline on oxygen consumption.

From Table 6 it also appears that the increase shown in the ergotamine-adrenaline tests is with considerable probability statistically significant. The most likely explanation is that the ergotamine did not completely inhibit the adrenaline effects. It might also be interpreted as an ergotamine effect, since the differ-

Table

The effect of ergotamine, adrenaline, ergotamine-adrenaline and The second

Guinea pig. No.	Weight gm	Adrenaline total dose γ	Ergotamine-tartrate total dose γ	Adrenaline tests			Ergotamine	
				Oxygen consumption cc/min			Oxygen consumption cc/min.	
				5—35 + 2 (35—65) min. after control injection	5—95 min. after adrenaline injection	Increase or decrease per cent	5—35 + 2 (35—65) min. after control injection	5—95 min. after injection of ergotamine
I.	500	10	10	7.1	7.7	+ 8	7.3	8.2
				6.6	6.7	+ 1	6.3	6.7
				—	—	—	—	—
II.	650	13	13	8.2	9.2	+ 12	7.6	8.3
				7.8	10.2	+ 31	7.4	6.8
				—	—	—	—	—
V.	550	11	11	7.0	7.4	+ 6	10.8	10.6
				7.5	8.9	+ 19	8.2	8.8
				—	—	—	—	—
VI.	550	11	11	7.4	7.7	+ 4	8.2	7.5
				8.6	9.0	+ 5	5.9	6.6
				—	—	—	—	—
VII.	500	10	10	8.5	8.6	+ 1	6.1	6.3
				6.7	6.7	0	7.8	7.8
				—	—	—	—	—

ence as between the ergotamine and the ergotamine-adrenaline tests may very well be due to chance.

As mentioned above, the increase found in the adrenaline-ergotamine tests is significant, and the difference between the adrenaline-ergotamine tests and the ergotamine-adrenaline tests is also statistically significant. This difference may be due to the fact that the inhibition only sets in after some time in the cases where ergotamine was given after adrenaline, whereas in those cases where ergotamine was given before adrenaline, the inhibition sets in immediately. That this will not be the case appears from what follows. As shown in Fig. 2 a distinct increase in oxygen consumption may be observed as early as 10 minutes after the adrenaline injection. Thus after 10 minutes a sufficient amount of adrenaline has been absorbed to effect an increase in oxygen consumption. If ergotamine is given 15 minutes before adrenaline,

4.

adrenaline ergotamine on the oxygen consumption of guinea pigs. test series.

tests	Ergotamine-adrenaline tests			Adrenaline-ergotamine tests		
	Oxygen consumption cc/min.			Oxygen consumption cc/min.		
Increase or decrease per cent	5—35+ 2 (35—65) min. after control injection	20—110 min. after injection of ergotamine = 5—95 min. after adrenaline	Increase or decrease per cent	5—35 min. after controlinjection	15—105 min after adrenaline = 5—95 min. after ergotamine	Increase or decrease per cent
+ 12	8.1	8.2	+ 1	7.0	7.1	+ 2
+ 6	7.1	8.6	+ 21	7.8	7.1	— 10
—	7.9	7.7	— 3	7.5	8.4	+ 16
+ 10	9.5	9.6	+ 1	8.2	10.0	+ 23
— 8	9.0	8.8	— 1	9.0	10.3	+ 14
—	9.7	9.2	— 5	8.3	10.7	+ 29
— 2	8.6	8.5	— 1	7.9	9.5	+ 20
+ 7	8.8	9.9	+ 13	7.6	8.1	+ 7
—	9.9	10.3	+ 4	8.2	8.8	+ 7
— 9	6.8	6.8	+ 1	9.2	10.8	+ 18
+ 12	9.0	8.5	— 5	6.9	9.1	+ 32
—	8.4	7.6	— 10	6.8	8.7	+ 28
+ 3	7.4	8.0	+ 8	7.2	8.3	+ 15
0	7.4	7.9	+ 7	7.8	7.7	0
—	7.3	8.1	+ 11	7.9	8.1	+ 3

it is fully inhibited. Therefore it will be possible to say that a sufficient amount of ergotamine is absorbed after 25 minutes to render possible an inhibition of the effect of adrenaline. If the oxygen consumption is determined 35—95 minutes after the injection of adrenaline in the adrenaline-ergotamine tests and the ergotamine-adrenaline tests, a complete inhibition ought to have taken place in both cases. 35—95 minutes after the injection of adrenaline the mean for the adrenaline-ergotamine tests and the standard error of the mean amounts to $+ 12.8\ \% \pm 3.79$, whereas the corresponding figures for the ergotamine-adrenaline tests are $- 2.7\ \% \pm 2.28$. The difference $+ 15.5\ \% \pm 4.43$, $t = 3.500$ and $P = 0.001 - 0.01$. Thus the difference is still statistically significant. From Table 6 it also appears that the sum of the effects in the adrenaline tests and the ergotamine tests does not differ from that in the adrenaline-ergotamine tests. Thus if ergot-

Table 5.

Statistical analysis of the values of table 4.

Variate	Mean deviation from the normal values per cent	Number of tests	Standard error of the mean	Degrees of freedom	t	P
Adrenaline tests...	+ 8.6	10	± 3.02	9	2.842	0.01—0.02
Ergotamine tests..	+ 2.9	10	± 2.48	9	1.169	0.2—0.3
Ergotamine-adrenaline tests	+ 2.7	15	± 2.02	14	1.340	0.2—0.3
Adrenaline-ergotamine tests.....	+ 13.6	15	± 3.06	14	4.268	< 0.001

Table 6.

Statistical analysis of all drug tests on guinea pigs.

Variate	Mean deviation from the normal values per cent	Number of tests	Standard error of the mean	Degrees of freedom	t	P
Adrenaline tests...	+ 12.0	24	± 2.22	23	5.397	< 0.001
Ergotamine-adrenaline tests	+ 3.47	29	± 1.296	28	2.677	0.01—0.02
Sum of Adrenaline tests and Ergotamine tests	+ 14.9	—	± 3.32	32	—	—
Difference between Adrenaline tests + Ergotamine tests and Ergotamine-adrenaline tests ..	+ 11.4	—	± 3.57	51	3.202	0.001—0.01
Difference between Adrenaline-ergotamine tests and Ergotamine-adrenaline tests	+ 10.1	—	± 3.34	42	3.024	0.001—0.01
Difference between Adrenaline tests + Ergotamine tests and Adrenaline-ergotamine tests	+ 1.2	—	± 4.52	46	0.266	0.7—0.8

Table 7.

The effect of adrenaline and ergotamine-adrenaline on cats.

Cat No.	Weight gm	Adrenaline total dose γ/kg	Ergotamine-tartrate total dose γ/kg	Adrenaline tests			Ergotamine-adrenaline tests				
				Oxygen consumption cc/min.		Increase or decrease per cent	Oxygen consumption cc/min.		Increase or decrease per cent	Oxygen consumption cc/min.	Increase or decrease per cent
				60—0 min. before injection	15—75 min. after injection		60—0 min. before injection	5—35 min. after injection of ergotamine		15—75 min. after injection of adrenaline	
I.	2100	100	50	16.1	22.2	+ 38	21.4	18.5	— 14	17.5	— 18
				17.3	19.9	+ 15	18 9	17.6	— 7	18.9	0
II.	1940	100	50	13.1	17.5	+ 34	17.5	19.2	+ 10	16.7	— 5
				17.1	22.5	+ 29	25.3	28.4	+ 12	23.6	— 7
III.	2210	100	50	22.7	24.2	+ 7	26.0	25.9	0	22.4	— 14
				20.9	22.7	+ 9	30.0	25.1	— 16	22.2	— 26
IV.	3020	150	75	28.0	30.3	+ 8	24.1	22.1	— 8	20.1	— 16
				23.9	29.8	+ 25	21.3	21.9	+ 3	18.3	— 14
V.	2230	110	67	26.1	28.5	+ 9	20.4	26.2	+ 29	16.3	— 20
				11.4	12.2	+ 7	16.6	18.0	+ 8	18.8	+ 13

amine is given before adrenaline, the increase of oxygen consumption is inhibited. If, however, ergotamine is given during a manifest increase of oxygen consumption after adrenaline no inhibition can be proved.

It was thought to be of interest also to make tests on the cat whether ergotamine inhibited the increasing effect of adrenaline on oxygen consumption. In order to secure a stronger effect of the adrenaline than that found in the tests on guinea pigs, 50 γ per kg of body weight was given intramuscularly. Of ergotamine-tartrate 25 γ per kg of body weight was given intramuscularly. The original intention was to use 5 animals, making 6 tests on each animal, viz. 2 tests with adrenaline, 2 tests with ergotamine-adrenaline, and 2 tests with ergotamine. 4 of the original 5 cats died from an intercurrent infection, however, before the ergotamine tests could be carried out, so they were replaced with others. The effect of ergotamine had been very similar on all the cats, wherefore the exchange was probably of no great importance. The tests were made in the following manner. After the animals had quitened down, 15—30 minutes after they had been put into the container, the normal oxygen consumption was recorded for 60 minutes. In the adrenaline tests the drug was injected in one hind leg, whereupon oxygen consumption was recorded for 15—75 minutes. In the ergotamine-adrenaline tests, ergotamine was given first, and the oxygen consumption was measured during 5—35 minutes. Then adrenaline was given 35—40 minutes after the ergotamine, and the oxygen consumption was recorded during 15—75 minutes. In the ergotamine tests the oxygen consumption was measured for 5—110 minutes. The injection of the drugs was effected without removing the animal from the container. As a rule the cats reacted but slightly to the puncture.

The results of the adrenaline and the ergotamine-adrenaline tests are assembled in Table 7, and those of the ergotamine tests in Table 8. The statistical analysis is to be found in Table 9. As shown in the tables, the normal oxygen consumption of the same animal varies considerably from day to day. The effects of the different drugs is, however, fairly uniform. In all cases adrenaline increases the oxygen consumption by 18 % on an average. Ergotamine has no certain effect during the first 30 minutes. The very high values for animal no. 9 may be due to motor unrest. 50—110 minutes after the injection, however, the oxygen consumption decreases by 13 % on an average. In the ergotamine-adrenaline tests, as in the ergotamine tests, the ergotamine has no definite effect during the first 30 minutes. After the adrenaline injection, the oxygen consumption decreases by 10 % on an average. As in the tests with guinea pigs, it has been investigated whether the sum of the effects of the adrenaline and

Table 8.

The effect of ergotamine on cats.

Cat No.	Weight gm	Ergotamine-tartrate total dose γ/kg	Ergotamine tests				
			Oxygen consumption cc/min.		Increase or decrease per cent	Oxygen consumption cc/min. 50—110 min. after injection	Increase or decrease per cent
			60—0 min. before injection	5—35 min. after injection			
IV.	3020	75	24.0	25.0	+ 4	22.1	— 8
			23.3	19.2	— 17	17.8	— 24
VI.	2650	67	17.2	18.7	+ 9	14.7	— 15
			17.9	17.8	— 1	15.1	— 15
VII.	2400	60	20.9	21.8	+ 4	17.3	— 17
			13.3	11.8	— 11	12.0	— 10
VIII.	2100	50	15.0	14.0	— 6	12.6	— 16
			15.6	19.5	+ 25	13.3	— 15
IX.	2800	60	19.2	24.7	+ 29	18.8	— 2
			18.0	28.0	+ 60	15.7	— 13

Table 9.

Statistical analysis of the values of table 7 and 8.

Variate	Mean deviation from the normal values per cent	Number of tests	Standard error of the mean	Degrees of freedom	t	P
Adrenaline tests...	+ 18.0	10	± 3.85	9	4.665	0.001—0.01
Ergotamine tests 5—35 min. after injection	+ 5.7	20	± 4.49	19	1.269	0.2—0.3
Ergotamine tests 50—110 min. after injection.....	— 13.4	10	± 1.83	9	7.341	< 0.001
Ergotamine-adrenaline tests	— 10.7	10	± 3.58	9	2.982	0.02—0.01
Sum of Ergotamine tests and Adrenaline tests..	+ 4.6	—	± 4.27	18	—	—
Difference between Adrenaline tests + Ergotamine tests and Ergotamine-adrenaline tests ..	+ 15.3	—	± 5.56	27	2.750	0.01

the ergotamine is divergent from the effect of the ergotamine-adrenaline, and the results are found in Table 9. The probability that the difference is due to chance, is only about 0.01. Thus also in the case of cats, ergotamine proves to inhibit the effect of adrenaline on oxygen consumption. No difference between the ergotamine and the ergotamine-adrenaline tests can be established. Hence the decrease of oxygen consumption in the ergotamine-adrenaline tests is an effect of the ergotamine and not an adrenaline reversal.

Discussion.

In calculating the effect of oxygen consumption after adrenaline, ergotamine and combinations of both, the indicated normal values have been taken as a starting-point. Probably these will not represent the basic metabolism, since the ainmals move about during the tests. However, since the animals also move during the drug tests. the effect is cancelled out by the motor activity. According to V. EULER and LILJESTRAND (*l. c.*) and MORIN (1943) the effect of adrenaline on the oxygen consumption decreases if the basic metabolism is increased. The absolute magnitude of the effect of the different drugs is, however, of less interest in this investigation than the difference in the effects. Part of the variation which is especially noticeable in the adrenaline tests will probably be due to differences in the motor activity during control tests and during drug tests. In order to reduce this variation it would be best to measure the oxygen consumption only during the periods when the animal is at rest, as has been proposed in certain quarters. This would, however, be impossible in an investigation of this sort, where the oxygen consumption must be compared at the same intervals after the injections of the drugs.

The effect of the control injection is surprisingly small. As already mentioned, the oxygen consumption becomes constant already 10 minutes after the injection and then remains constant for 55 minutes. Whether it returns to its original value after that interval is not shown by this series of tests. But this is actually the case (LUNDHOLM, unpublished). The effect of the injection is further reduced owing to the fact that the animals get accustomed to the prick of the needle. This proves how necessary it is that the control injections should be made simultaneously with the drug injections, if the injection effect is to be

taken into account. In view of the fact that the animals get used to the puncture, it is best to use the same animal.

After the administration of 20 γ per kg of body weight of adrenaline the oxygen consumption of guinea pigs increases by 12 % on an average during 90 minutes. In the individual tests the percentages may vary from 0 to +37 %. During shorter periods, however, the increase may amount to as much as 75 %. For the cat, the corresponding figure after 50 γ of adrenaline is + 18 % on an average within variation limits of + 7—+ 38 %. On an average the increase for guinea pigs amounts to about 20 %, during the first 30 minutes. This value tallies well with the figures in the previously quoted works by v. EULER and LILJESTRAND (*l. c.*) for tests on man, and with those of MOERLOOSE (*l. c.*) for tests on the dog. The great variations in the results of the tests may be partly due to a varying motor activity during control tests and drug tests. If the variation in the adrenaline tests was due solely to that, the dispersion would be the same in all the tests. If, however, the standard deviation and its standard error for all adrenaline tests (= adrenaline and adrenaline-ergotamine tests) is calculated, the value 11.24 % ± 1.02 is obtained. The corresponding figures for tests without adrenaline effect (= ergotamine and ergotamine-adrenaline tests) are 6.95 %±0.73. The difference is 4.29 % ± 1.25, $t = 3{,}432$, $P = 0.001$. The standard error of the standard deviation has been calculated according to the formula $\pm \frac{\mu_4 - \mu_2^2}{\mu_2 n}$ where μ_2 and μ_4 are the second and fourth moment about the mean respectively, and n the number of determinations. This formula should be applied if the sampling distribution is not normal (YULE and KENDALL 1947). Thus it must be assumed that the effect of adrenaline varies from one test to another. In Table 10 the variation "between" and "within" guinea pigs has been calculated for the adrenaline tests according to the methods of analysis of variance (BONNIER-TEDIN, 1940). The variation "between" guinea pigs is no larger than that "within" guinea pigs, and no obvious individual sensitivity for different animals can be established. This does not mean that such a difference does not exist, but it is probably so small that it may be ignored.

In the case of guinea pigs, ergotamine in doses of 20 γ per kg of body weight has no definite effect on oxygen consumption. In the case of cats, however, it decreases by 13 % on an average

Table 10.

Statistical analysis of the variance in the adrenaline tests on guinea pigs.

Variance	Degrees of freedom	Sum of Squares	Mean Squares	v^2	P
Between guinea pigs	6	770.0	128.3	$\frac{128.3}{114.1} =$	> 0.2
Within guinea pigs .	17	1940.0	114.1	$= 1.124$	
Total	23	2710.0	—	—	—

after 25 γ per kg of body weight 50—110 minutes after the injection. The decline in oxygen consumption tallies with the results of the investigations by ABDERHALDEN and WERTHEIMER (1927) on the rat, those of MARINE, DEUTCH and CIPRA (*l. c.*) on the rabbit, and those of MICHAEL, BENDESCU and VANCEA (1928) on man. Cats, however, seem to be specially sensitive to ergotamine.

The inhibiting effect of ergotamine on the oxygen consumption after adrenaline, shown in tests on guinea pigs and cats, conforms with v. EULERS (*l. c.*) findings in tests with tissues. The result also tallies with the general experience that ergotamine inhibits adrenaline, especially its stimulating effects. This is the case only if ergotamine is given before adrenaline. If ergotamine is given during a manifest increase of the oxygen consumption after adrenaline, no inhibition can be traced. By way of speculation, one of the following three explanations can be suggested:

1. Ergotamine does not inhibit an adrenaline effect already in progress. This assumption is, however, negatived by an investigation carried out by MASUDA (1925), who, during the course of adrenaline perfusion in the hind leg of the frog, found that ergotamine still caused vasodilatation. Also during the course of continuous intravenous infusion on the cat, we have found that ergotamine can nullify the effect of adrenaline on the blood pressure.

2. If ergotamine is given after adrenaline a larger dose is required to inhibit the effect of adrenaline than if ergotamine is given before adrenaline. We have been unable to find any quantitative investigations as to whether this is the case.

3. The increased oxygen consumption after adrenaline is not a direct effect of the adrenaline but the result of an adrenaline effect which had occurred before a sufficient amount of ergotamine had

been absorbed to inhibit the adrenaline. It is at present impossible to say which of these explanations is the right one.

The failure of ergotamine to inhibit the increasing effect of adrenaline on oxygen consumption, if it is given after the adrenaline, might explain why CAPO (*l. c.*) and YOUMANS, TRAUBE, BUVINGER (*l. c.*) did not succeed in showing the inhibiting effect of ergotamine, when they gave ergotamine simultaneously with or after adrenaline.

Summary.

The effect of adrenaline and ergotamine on oxygen consumption, as well as that of combinations of the two, has been investigated for guinea pigs and cats.

1. 5—10 minutes after the injection of 1 cc of 0.9 percent NaCl-solution the oxygen consumption increases moderately, but this increase declines rapidly with an increasing number of injections, because the animals get accustomed to the puncture.

2. In the case of guinea pigs 20 γ per kg of body weight of adrenaline increases the oxygen consumption by 12 % on an average 5—95 minutes after subcutaneous injection. The effect of adrenaline varies considerably from one test to another. The variation is the same in repeated tests on the same animal as in tests on different animals.

3. 20 γ per kg of body weight of ergotamine-tartrate injected intramuscularly has no definite effect on oxygen consumption of guinea pigs.

4. 20 γ per kg of body weight of ergotamine-tartrate given 15—35 minutes before 20 γ per kg of body weight of adrenaline nearly completely inhibits the increasing effect of adrenaline on the oxygen consumption.

5. If the same dose of ergotamine-tartrate is given 10 minutes after adrenaline no inhibition can be traced. Alternative explanations of this fact are discussed.

6. On the cat 50 γ per kg of body weight of adrenaline injected intramuscularly increases the oxygen consumption by 18 % on an average, 15—75 minutes after the injection.

7. On the cat 25 γ per kg of body weight of ergotamine-tartrate injected intramuscularly has no definite effect upon the oxygen consumption during the first 30 minutes. 50—110 minutes after

the injection, the oxygen consumption decreases by 13 % on an average.

8 In the case of the cat, too, ergotamine given 35 minutes before adrenaline completely inhibits the increasing effect of adrenaline on the oxygen consumption.

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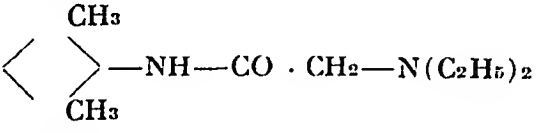
p. 28 l. 29 

p. 28 l. 40 clinically
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p. 33 l. 8 520 mμ
p. 33 l. 14 1.17 in 5 ml.
p. 46 l. 5 carotid
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p. 70 l. 30 Rothstein and Enns (1946)[3]) show that ethyl alcohol has the same ability.

ASMUSSEN, ERLING, HALD, JENS, JACOBSEN, ERIK and JØRGENSEN, GUNNAR; (Copenhagen): **Observations on respiration and circulation of human individuals hypersensitive to alcohol.**

In a communication at this congress by Hald, Jacobsen and Larsen is described the effect of small doses of ethylalcohol after intake of tetraethylthiuramdisulphide (antabus). In the present investigation we have examined the effect of alcohol on circulation and respiration in normal resting human subjects before and after treatment with antabus. Half an hour after intake of 60 ml aquavit (43 vol. % alcohol) no effect was seen on pulse rate, cardiac output, dead space, ventilation and CO_2 % in alveolar air. When the individuals were treated with 1.5 g antabus the previous day, the same dose of alcohol excerted a marked increase in pulse rate and a slight increase in cardiac output. No decisive effect on blood pressure was seen. There was observed a marked increase in ventilation combined with a decrease in alveolar carbon dioxide. A moderate increase in the volume of the dead space was also observed.

The observed effects correspond well to the effect of acetaldehyde as described in animal experiments by Handowsky (1934).

ASMUSSEN, ERLING and NIELSEN, MARIUS; (Copenhagen): **The initial changes in ventilation on transition from rest to work and from work to rest.**

The mechanism involved in the regulation of the ventilation has been studied under different conditions, notably the transition from rest to work and from work to rest.

In the former kind of experiments it was found that the ventilation at the onset of work begins to increase immediately, before any changes in the composition of the blood irrigating

the respiratory centre can possibly have occured. As a consequense the alveolar pCO_2 undergoes a decrease during the first 0.3 to 0.6 min. of work. These results are in conformity with the results of Krogh and Lindhard (1913) and show that the impulses responsible for the increase must be of nervous origin. With electrically induced work the increase in ventilation was found to progress in the same way (comp. Krogh and Lindhard 1917).

The ventilation in the first 0.3 min. of work is plotted against the rate of work, and the curves show that this initial ventilation increases with the severity of work, but that the curves are flattening out with the heaviest work.

From these results it is concluded that the initial increase in ventilation is brought about by proprioceptive impulses arising in the mowing limbs and integrated so as to produce an increase in the excitability of the respiratory centre correlated to the mechanical tensions set up by the working muscles. This is in accordance with our earlier results from the steady state of work and with the results of Comroe and Smith (1943).

The flattening out of the curves at the heavy work is in accordance with the earlier observations of the authors (1946) viz. that the reflexes are able to increase the ventilation only up to a certain limit and that the further increase in ventilation is brought about by substances produced in the working muscles.

At the transition from work to rest the ventilation begins to fall immediately, i.e. before any changes in the composition of the arterial blood have occured. This is assumed to indicate that at the stopping of work a factor of nervous origin, that has helped in keeping the ventilation increased, now suddenly disappears, and it is assumed that this factor must have been the proprio-ceptive impulses from the moving limbs.

The results are taken as further evidence for the assumption, that proprio-ceptive impulses from the moving limbs play an important rôle in the regulation of the ventilation during light work.

ASTRUP, T. and PERMIN, PER M.: **Fibrinolytic enzymes in blood.**

It is known that the blood under certain circumstances is able to show fibrinolytic activity (proteolytic activity), but the mechanism of this process has only recently become clear.

It has been shown that the effect of haemolytic strepto-

cocci and chloroform is due to the activation of a proenzyme (plasminogen, profibrinolysin) in the plasma to the active enzyme (plasmin, fibrinolysin), e.g. L. R. Christensen & C. M. MacLeod (J. Gen. Physiol. 1945, 28, 559) and E. C. Loomis, C. George & A. Ryder (Arch. Biochem. 1947, 12, 1) The activator in streptococci is called streptokinase.

In the coagulation of blood it has also been found that proteolytic enzymes occurred, but Hudeman (Kolloid- schr. 1940, 92, 189) has shown that these had nothing to do with the thrombin, which alone is without any fibrinolytic effect. Also in the preparation of prothrombin (T. Astrup & S. Darling, Act. Physiol, Scand. 1943, 5, 97) it was found that the solutions contained proteolytic enzymes, which in course of a short time spoiled the prothrombin, and it was suggested that these enzymes could have been formed by means of an activiting process similar to the formation of thrombin from protrombin. A mechanism similar to this, was later suggested for the fibrinolytic process, noticed in tissue culture (A. Fischer: Nature 1946, 157, 442).

During investigations on this formation of proteolytic enzymes, it was found that the tissue cells contain an activating substance (fibrinokinase) which is able to activate the proenzyme active (T. Astrup & Per M. Permin: Nature 1947 159, 681) It was found impossible to extract the activating substance from the tissue protein, but by means of acetone and ether a dry powder war prepared, which contained the activating substance (Per M. Permin: Nature 1947, 160, 571). The properties of the fibrinokinase and the active enzyme have been examined and compared with the active enzymes formed by means of chloroform and streptokinase (Per M. Permin: to be published) Later it was found, that human blood possibly contains two different proenzymes (T. Astrup & Per M. Permin: Nature 1948, 161, 689) only one of which is able to be activated by fibrinokinase. It was shown, that the streptokinase used in Copenhagen, only could activate the proenzymes in human blood, but not the proenzymes in bovine blood. Meanwhile, later investigations carried out in Toronto (T. Astrup, J. Crookston & A. MacIntyre) in which a preparation of streptokinase of L. R. Christensen, New York, was used, have shown, that also an activation of the proenzyme in bovine blood was possible, but only to the same extent as with the use of fibrinokinase. The proenzyme in human blood was activated to a greater extent by means of streptokinase, perhaps due to the presence of two different proenzymes in the human blood.

BARANY, ERNST and KINSEY, V. EVERETT, Uppsala: **The rate of flow of aqueous humour and its significance for intra-ocular fluid problems.**

The rate of flow of aqueous humour in individual rabbits has been measured by a new method. The principle is to introduce a slowly diffusing substance into the aqueous from the blood and then determine the rate at which the substance disappears from the aqueous after the concentration in the plasma has fallen to such a low level that essentially no additional test substance enters the eye. P-aminohippuric acid is a suitable test substance. Twenty-three determinations gave a median half-life of p-aminohippuric acid in the aqueous of 49 minutes (rate constant $K_{out} = 0.0141$).

If there were no loss of p-aminohippuric acid by any means other than flow, i.e. if it entered the aqueous only by secretion and left only by flow, the rate of disappearance and rate of flow, both expressed on a percentage basis, would be the same. If, however, all, or some of the substance enters the eye by diffusion, then some also will leave by diffusion and the rate of flow will be less than that indicated by the rate of disappearance. The lower limit of the rate of flow can be determined from the rate of disappearance and the ratio of concentration of the test substance in the aqueous compared with that in the plasma under steady state conditions according to the equation

$$\frac{K_{diffusion}}{K_{diffusion} + K_{flow}} = \frac{\text{Conc. aqu.}}{\text{Conc. pl.}}$$

where $K_{diffusion} + K_{flow} = K_{out}$. The steady state ratio conc.aqu/conc.pl. is 0.24 for free p-aminohippuric acid. Using this value and $K_{out} = 0.0141$ the lower limit for rate of flow corresponds to a half-life of 62 minutes ($K_{flow} = 0.0112$).

The half-life for flow under strictly physiological conditions thus lies between 49 and 62 minutes. Lacking knowledge of the mode of entrance of p-aminohippuric acid it is not possible to give a more exact value from the present data. From these results, however, and the fact that sodium has a half-life of approximately 50 minutes in the aqueous it follows that at least 70 per cent of the sodium enters the aqueous by secretion in the rabbit. Similar estimations can be made for other substances as soon as their half-life in the aqueous humour is known.

BERGER, CURT; Buenos Aires: **Some experiments on the width of symbols as determinant of legibility.**

The legibility of letters (printed type) has not only a considerable practical interest for the printing industry, but also for the understanding of some problems of optical physiology of the retina and the central nervous system, especially because Snellen's test-carts are used for measurements of visual acuity.

Although both aspects have led to considerable practical and theoretical research, the basic factors which determine the legibility of single letters (and thereby influence reading) as width, height and stroke-width of symbols have not yet been examined. Since these factors must have a physiological basis, the influence of width of two symbols, namely 0 and 5 were investigated with three different methods: (1) Threshold-determinations of the distance at which the symbols disappeared (with constant medium intensity, 22 foot-candles). (2) With the Luckiesh-Moss visibility meter. (3) Threshold-determinations of minimum intensities at which the symbols became illegible (at a fixed distance of about 2.0 m from the eye).

Results: (a) Method 1 and 3 were in good agreement, while the applications of method 2 were limited.

(b) the results with the other 2 methods are: With increase of width increases legibility. This increase is not linear and not the same for both symbols. While the symbol 0 with small width (1.5 mm) is less legible than the corresponding 5, its legibility increases faster than the other. The two curves intersect and the legibility of the 0 continues to increase faster. The increase of the 0 is between 150 and 300 %, while for the 5 it is only 20 % to 30 %, for changes of width between 1.5 and 4.15 mm and 6 mm height. (4) The results can be explained on the basis of the resolution threshold for two adjacent black borders which are different for the two numerals.

(1) LUCKIESH, MATHHEW: *Light, vision and seeing.* 1944. D. van Nostrand Co.

(2) BERGER, C.: *Skand. Arch. Physiol.* 1935. *71.* 173.

BING, JENS; Copenhagen: A preliminary report on the effect of partial corticectomy or partial medullectomy of the kidneys in different mammals.

Using a resorbable hemostatic, a danish gelatine sponge preparation («Spongostan»), it was found, that it is possible to perform partial corticectomy or partial medullectomy on the kidneys of different mammals. As it was thought, that

it by help of such operations might be possible to distinguish between the function of periferal and central nephrons and get other informations about kidney function, the operations were performed on rats, cats, dogs and rabbits.

It was found that it is possible to keep the rats alive, and in such animals diuresis, chloride excretion, blood urea and blood pressure were studied. In studies on dogs and rabbits it was found, that they could be kept alive after the partial corticectomy, which was performed on one or both sides, and in these animals kidney function showed significant differences from the normal. Cats seem to be more sensitive to the results of the operation.

Preliminary studies were made on the microscopical picture of kidneys after different degrees of the operations.

BONSDORFF, EVA; Helsinki: **On the humoral mechanism in anoxic polycythaemia.**

In a previous paper Bonsdorff & Jalavisto (in press) have shown, that in the plasma of chronically anoxic organisms (i.e. in patients with congestive failure) as well as in the plasma of acutely anoxic rabbits, substances are present, which promote erythrocytosis, when injected into normal rabbits.

To investigate the site of formation of the active substances, heparinized blood from normal rabbits was exposed to lowered atm. pressure (200—400 mmHg) for 4 hours. The blood was centrifuged, and 3 cc. of the plasma was injected intraperitoneally into 6 normal rabbits. In each rabbit the injection was followed by an erythrocytosis from 0,5 to 0,8 mill., and a reticulocytosis of about 6—7 %. The reaction lasted 2—4 days. In control experiments samples of the same blood were kept in normal atm. pressure for 4 hours and the plasma injected into normal rabbits. The injection gave no response. Plasma, when exposed to low pressure gained, however, no erythropoictic activity.

The results show, that erythropoietic substances appear in the plasma, when whole blood is exposed in vitro to low atm. pressure. Plasma, when exposed, remains inactive. Further investigations on the subject are in progress.

BOOTHBY, WALTER M., LUNDIN, GUNNAR and HELMHOLZ, H. FREDERIC, Jr.: **Agaseous nitrogen elimination test to determine pulmonary efficiency.**

A subject or patient breathing air through a valve and mask, begins breathing oxygen from a closed circuit with a Krogh type spirometer. A period of washing out of nitrogen from the lungs and body takes place. Increase in nitrogen concentration is followed by a continously sampling and indicating Lilly nitrogen meter. When plotting the volume of nitrogen accumulated in the spirometer system against time two distinct phases can be separated. A first phase during which the lung-gases are coming into equilibrium with the spirometer gases, a second during which only the nitrogen from the blood and tissues is passing into the system. There is a distinct change in slope or a knee at the end of the first phase, the final part of the curve (representing the tissue nitrogen) being a straight line to the end of the observation perod which is usually 30 minutes.

Such a test combined with measurements of vital capacity gives us the following informations.

1) Wash out time for nitrogen in the lungs during basal conditions.
2) Functional residual volume.
3) Total capacity and residual volume.

Any changes in the factors which may change the balance between mixing and diffusion either locally or in the entire lung will be reflected in the wash out time. Under standard and basal conditions the various components of the lung volume and the wash out time are reproduced within narrow limits. In normal individuals the wash out time varies between 2.5—3.5 minutes.

Besides normal subjects, a preliminary series of patients have been studied, and we have found that the results obtained appear to correlate with the clinical diagnosis and that the test can provide the clinician with significant informations.

BUCHTHAL, F., DEUTSCH, A., KNAPPEIS, G. G. and MUNCH-PETERSEN, A.; Copenhagen: **Chemical aspects of the volume constriction of actomyosin threads by adenosine triphosphate.**

Three times reprecipitated actomyosin contains appreci-

able amounts of adenine, ribose and phosphorus, which cannot be removed by repeated washing or dialysis, except after denaturation of the protein. Treatment of actomyosin threads with adenosine triphosphate (ATP) in amounts causing maximum volume constriction, results in a considerable increase of the adenine, ribose and phosphorus content of the protein. At least one third of the total phosphorus — corresponding to somewhat more than one mole of phosphate for each mole bound adenine — appears as orthophosphate after denaturation; consequently it must be present as highly labile phosphate in the protein. The rest of the phosphorus corresponds to approximately two moles of phosphate per mole of bound adenine and is nearly equally distributed between the pyrophosphate and non-labile phosphate fractions. The treatment of actomyosin threads with ATP results thus in a phosphorylation of the protein under simultaneous uptake of adenine nucleotide, the final nucleotide content corresponding in its adenine/phosphorus ratio to adenosine diphosphate (ADP).

Actomyosin threads with low and high adenosine triphosphatase activity give the same results after treatment with ATP; volume constriction is likewise independent of the triphosphatase activity of the threads.

In the presence of magnesium salts the volume constriction caused by ATP is increased (Szent-Györgyi); we find that the accompanying chemical changes in actomyosin are affected in the same direction.

When ATP is substituted by equimolar amounts of ADP, adenylic acid or inosine triphosphate (ITP), and by four times larger amounts of orthophosphate, pyrophosphate or triphosphate, the actomyosin threads remain unchanged. No volume constriction occurs and no chemical changes can be observed, although both ITP and inorganic triphosphate are acted upon by the adenosine triphosphatase associated with actomyosin.

Our results indicate the independence of the observed chemical changes of the adenosine-triphosphatase activity of actomyosin. On the other hand they show the close parallelity of volume constriction and chemical changes.

No volume constriction is produced by ATP in actin-free myosin threads, the chemical changes however, observed in actomyosin threads, occur in threads of actin-free myosin as well. Thus, the physical changes in actomyosin on interaction with ATP, are bound to the presence of actin, whereas the chemical changes, localised to the myosin moiety of the protein, are produced in the absence of actin as well.

BUCHTHAL, FRITZ and KAISER, E.: The mechanics of isotonic contraction in relation to minute structure.

When investigating minute-structural properties of a muscle fibre, especially their correlation to physiological reactions, the analysis of mechanical properties, though indirect, is still an important tool which has certain advantages compared with a direct assay by X-ray diffraction or electron-microscopy.

Dynamic and static mechanical properties are examined by means of a recording system which gives an undistorted record of isotonic contractions in the isolated fibre. It consists of a light moving coil system. By introducing direct or alternating current, constant loads and alternating loads of different frequencies can be applied to the fibre. The resulting alterations in length are recorded optically or transmitted photoelectrically to the y-plates of a cathode-ray oscillograph. A Lissajou figure is produced by simultaneous vertical deflections proportional with the instantaneous value of the current in the coil, giving an exact correlation between the periodic force applied and the resulting changes in length. Thereby the dynamic elastic stiffness and $\frac{\text{elastic stiffness}}{\text{viscous stiffness}}$ ($\text{tg}\,\varphi$) are determined at resonance frequency.

The length-tension diagram for isotonic contraction lies below the curve of isometric maxima, apart from loads $< P/Po = 0{,}3$, where the curves coincide. Shortening velocity (v_c) decreases with increasing load (Hill); in the isolated fibre v_c can attain double the values found in total muscle, indicating a higher efficiency in the former. The velocity of relaxation (v_c) is less well defined than v_d, it varies less with load and more with temperature. Under all conditions investigated (temperature, load, fatigue) shortening in a twitch relative to a tetanus is found to be a function of the corresponding tetanic $\frac{v_d}{d_c}$.

Elastic stiffness at rest increases proportionally with load and falls with increasing temperature 1 per cent/°C. During isotonic contraction at 0° C. it exceeds resting values by up to 100 per cent. The difference is less at higher temperature. In contrast to rubber and myosin threads, in muscle stiffness decreases with increasing oscillation amplitude.

Changes in stiffness produced by contraction, temperature and oscillation amplitude decrease with higher loadings.

$\operatorname{tg} \varphi \frac{\text{(elastic stiffness)}}{\text{(viscous stiffness)}}$ is approximately 1,0 (0° C.) and contraction $\operatorname{tg} \varphi$ decreases. Like elastic stiffness, changes in viscous stiffness are reduced with increasing load.

A muscle fibre exhibits apart from kinetic elasticity characterizing high elastic substances deformations due to transition in chemical bond structure. Under isotonic conditions neither crystalline nor kinetic elasticity can account for the observed alterations in mechanical properties produced by contraction, temperature, load and oscillation amplitude. Minute-structural elements are considered to consist of links each alternating between long (1) and short (s) modifications. The probability for an $1 \rightarrow s$ transition initiated by thermal agitation is increased when the load is reduced and vice versa. Contraction should consist in a fixation of short modifications as they arise. The smaller probability for $1 \rightarrow s$ transition at higher loads could explain the reduced shortening velocity.

BUDOLFSEN, SVEND ERIK; Aarhus: **The Absorption of Glucose from the Large Intestine.**

The experiments were made on white rats, anesthetized with urethane. The rats were given water, but no food for 48 hrs.

The large intestine was isolated by means of ligatures at the ileocaecal place and at the sigmoid colon. At the same time a loop of the small intestine was isolated from the ileocaecal place and 20 cm proximal. Both loops were washed out thoroughly by means of 37° warm 0,9 % NaCl-solution. The accurately measured amounts of the glucose-solution were then put into the loops. At the end of the experiment the animal was killed. The loops of intestine were removed and the contents washed out with water. The glucose was estimated by the method of Hagedorn-Normann-Jensen.

There has been found absorption of glucose from the large intestine whether the solutions were hypo-, iso- or hypertonic. The amount absorbed depended on the concentration of glucose in the incubated solutions, but the ratio $\frac{\text{absorbed glucose}}{\text{incubated glucose}}$ was rather constant — about 20 % in 1 hour.

In the small intestine the amount of glucose absorbed also depended on the concentration of glucose in the incubated solutions, but the ratio $\frac{\text{absorbed glucose}}{\text{incubated glucose}}$ was diminished, when the concentration of glucose in the incubated solutions was increased.

Phlorhizin (1 %) in the incubated glucose-solution diminished the absorbed amount greatly in the small intestine, but had only a dubious effect on the absorbed amount of glucose in the large intestine.

From the caecum of rabbits it was shown that a glucose-solution with a lower sugar concentration than the blood can be absorbed without being concentrated to the blood sugar level, when the infusion-solution had been made isotonic with the blood with NaCl. The same experiment has not been possible on rats owing to the small infusion-volume.

BØE, JENS (Oslo): **The colloidal gold test.**

CARLSTEN, ARNE, FOLKOW, BJØRN, HAEGER, KNUT, KAHLSON, GEORG — WICKSELL, FINN, Lund: **Experiments on Reactive Hyperaemia** (communicated by Georg Kahlson).

The factors involved in eliciting vasodilatation in ischaemic tissues are as follows.

1. Nervous elements and reflexes are not fundamentally engaged since in our experiments this type of vasodilatation occurs in animals where vasodilators of all known types have degenerated after sympathectomy and section of antidromic vasodilators in the dorsal roots.

2. Mechanical factors as represented by the pulsatile pressure on the walls of the blood vessels are engaged in the initial state of vasodilatation during reactive hyperaemia.

3. Chemical factors are responsible for the prolonged, second state of vasodilatation during reactive hyperaemia. The physiological properties of lymph as related to this problem have been investigated.

CLEMEDSON, CARL-JOHAN; Stockholm: **Some effects of shock waves from detonating high explosives on respiration and circulation.**

Earlier investigations on blast injuries which is the syndrom of injuries, caused by the shock wave from a detonating charge of high explosive, have furnished us with a fairly clear picture of their clinical and pathological aspects. On the other hand, the influence of shock waves on the physiological functions is very incompletely known.

The author has studied the effects of detonations on respiration and circulation in rabbits exposed to charges of TNT (Trinitrotoluene, trotyl) detonated in open field and in a specially constructed detonation chamber. Electrocardiographic recordings have been taken continuously before, during and immediately after the detonation. Simultaneously, respiration has been registered with a thermo-electrical method. In some animals the blood pressure has been followed for several days with determinations of the pressure in the central artery of the ear according to the method deviced by Fahr.

The author has tried to correlate the degree of influence on respiratory and cardiac activity with the degree of injury to the lungs (haemorrhages and emphysema) and with the physical properties of the shock wave, chiefly its maximum pressure and impulse (timeintegral of pressure).

Exposition to small pressures which cause only slight pulmonary injuries is followed immediately by an increase in respiratory rate sometimes to several hundred per cent. In cases with high pressure and impulse values the detonation is followed by an immediate respiratory stand-still with the lungs in an inspiratory position, that might last from several seconds to minutes or until the death of the animal.

Cardiac activity is impaired only when the animal is exposed to shock waves of high pressure. The most constant alteration in the electrocardiogram is a transient, but very marked bradycardia. Other abnormalities e.g. heart block, bigeminy, ventricular, fibrillation, etc. may be seen. The influence of vagotomia has been studied.

Shock seems not to be a common feature in uncomplicated blast injury, but in some cases circulatory collapse may be the immediate cause of death.

CLOSS, KARL; Oslo: **Investigations on lipoids in various clinical conditions.**

Determinations of the serum lipoid content in a variety of clinical conditions do not allow any definite conclusions as to the underlying disturbances of lipoid metabolism. Some of the findings are discussed somewhat more in detail. In the

acute stage of acute hepatitis the total cholesterol content of the serum (TK) is either raised or lowered, while the cholesterol ester fraction (BK) always is redueed. The total fatty acid content (TF) is high in all eases, the highest figures being found in the acute stage of the disease. Also the lipid-phosphorus content (LP) is above normal. In obstructive jaundice the changes observed are almost identical. An explanation of these findings is proposed. In lipoid nephrosis similar changes are observed, with the exeeption of a normal ratio BK/TK. In cirrhosis of the liver as a rule all the lipoid fractions are lowered, but here, as in jaundice, the ratio BK/TK is below normal. The lowest values are eneountered in acute atrophy of the liver, where the BK fraction almost disappears. The different forms of steatorrhea are aecompanied by low figures for all the fractions, the ratio BK/TK being normal though. Characteristic of cases of «ventriculus operatus» are the high TF values, besides almost normal figures for BK, TK and LP. In adiposity the figures lie towards the upper, and in edema towards the lower normal limit. In Cushing's syndroma the values are high in all the fractions, especially TF and LP. In a case of an adrenal cortical tumor, where excessively large amounts of 17-ketosteroids were found in the urine, extremely low values for BK and TK were found. The values became normal shortly after removal of the tumor. The «chylus» in a case of ascites chylosus in a girl aged 7—14 months is characterized by a low cholesterol content, and high figures for neutral fat, and to a lesser degree for LP. Some implications with respect to the theory of lipoid absorption are discussed.

DAM, H.; Copenhagen: **The effect of feeding highly unsaturated fatty acids in the absence of vitamin E, and peroxidation of fat in vivo.**

During the study of the vitamin K-deficiency disease in chicks certain symptoms were found which later appeared to be due to the ingestion of liberal amounts of highly unsaturated fatty acids in the absence of vitamin E. One of these symptoms is the so called exudative diathesis (Dam & Glavind 1938, 1939, Dam 1944) which manifests itself by the exudation of blood plasma from the capillaries in adipose tissue. Another symptom is encephalomalacia, a cerebral disease which was first studied by Pappenheimer & Goettsch 1934. Its relation to vitamin E was shown by Dam, Glavind, Bernth & Hagens 1938, the relation to highly unsaturated fatty acids by Dam 1944. In attempts to develop similar

symptoms in rats a third symptom was found, viz. a brown discoloration of the depot fat (Dam 1944), which is also due to the same two causes. A fourth symptom, viz. depigmentation of the incisors in rats had been shown by Davis & Moore 1941 to be due to the lack of vitamin E. Granados & Dam, 1945, showed that fat, and Dam & Granados, 1945, that highly unsaturated fatty acids are necessary for the development of this symptom. These observations gave rise to a series of investigations on the possible role of an abnormal oxidation of highly unsaturated fatty acids in the affected tissue as cause of, or at least as an accompanying phenomenon in the afore mentioned symptoms (Dam & Granados, 1945b). The relation between vitamin E and antioxidant effect was already studied by Olcott & Mattill 1936. While the experiments here reported were going on other authors described the formation of a brown pigment i liver, lymph nodes and other organs in connection with experimental liver cirrhosis in rats due to the lack of cholin. Gyorgy & Goldblatt, 1942. Endicott & Lillie, 1944, named this pigment «C e r o i d», and it was later found that its development was also caused by the feeding of highly unsurated fatty acids in the absence of vitamin E. Our investigations show that the brown discoloured depot fat in rats contains considerable amounts of peroxides as determined by the methods of King, Roschen & Irwin, 1933. However, in chicks peroxidation of the adipose tissue was just detectable when the exudative diathesis began and no relation between the peroxidation value and the severity of symptoms could be found (Dam & Granados, 1945).

Substances which may exert a sparing action on vitamin E when ingested in large amounts (ascorbic acid 0.5 % of the diet or Nordihydroguaretic acid, same amount) can delay the development of the exudative diathesis. These investigations have branched out in several directions, namely to the antioxidant and fat oxidation problems in general, seen from the biological as well as from the industrial points of view. Some of these investigations will be discussed in the papers by J. Glavind, S. Hartmann, and H. Granados.

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VON DÖBELN, WILHELM; (Stockholm): **Surface decompression in deep diving.**

The stage decompression method of Haldane to avoid decompressionsickness in diving is accepted all over the world. A disadvantage with this method in deep diving is the long decompression times, during which the diver has to stay in the water. It would be more convenient if the diver immediately after the time on the bottom could be brought to the surface and the decompression could be performed in a recompression chamber. After a great number of experimental dives Hawkins and Shilling calculated the figures for the saturation with nitrogen where this is possible.

Tables for surface decompression in dives have been calculated by the author with regard to the figures given by Hawkins and Shilling. These tables have been used in several hundred dives to depths of 35—75 meters in the Swedish Navy with good results.

EMMELIN, N. G. and MACINTOSH, F. C.; London: **Some conditions affecting the release of acetylcholine in sympathetic ganglia and skeletal muscles.**

The theory of acetylcholine as a synaptic transmitter was founded on the demonstration (Feldberg and Gaddum 1934) that acetylcholine appears in the effluent from a perfused ganglion when its preganglionic fibres are stimulated. A parallel observation (Dale, Feldberg and Vogt 1936) is the basis of the theory that acetylcholine is the transmitter at the neuromuscular junction: in this case the effluent from a perfused muscle was found to contain acetylcholine only when the motor nerve suppying the muscle was stimulated. On the other hand when the ganglion is isolated as for perfusion but is allowed to retain its natural blood supply acetylcholine appears in much smaller quantity, if at all, in the venous blood leaving the ganglion, even when massive doses of eserine have been

administered to the animal (cf. MacIntosh 1938). Similarly Dale, Feldberg and Vogt (1936) were unable to demonstrate the presence of acetylcholine in the venous blood from a muscle retaining its normal circulation. In their successful experiments the perfusion fluid was eserinized Locke's solution (pH about 8.5) and the perfused muscles always became oedematous very rapidly. In the present experiments we have perfused the superior cervical ganglion or the tibialis anticus muscle with fluids of different composition: a modified Locke's solution, containing a phosphate buffer of pH about 7.4; heparinized cat's plasma; and defibrinated blood. The solutions contained either eserine, di-isopropylfluorophosphate or tetraethylpyrophosphate. Under all these different conditions acetylcholine could be detected in remarkably constant amounts in the venous effluent on stimulation of the preganglionic fibres of the ganglion or the motor nerve of the muscle; we think that this is strong evidence that the release of acetylcholine is a genuinely physiological phenomenon.

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ENGBÆK, LISE; Copenhagen: **Neuromuscular Transmission in Man.**

The results obtained in recent years with close arterial injection of acetylcholine into human muscle are in disagreement with the effect of injection of small amounts of this substance into mammalian muscle. While in the latter it produces a short tetanus-like contraction (Brown et al. 1936), in normal human muscle a paresis has been observed following the injection of comparatively large amounts, which however in patients with impaired neuromuscular transmission produced a violent motor response (Harvey et al. 1941). This difference was considered to indicate an increased acetylcholine sensitivity of e.g. myasthenic muscle. Since the effect of small amounts of acetylcholine apparently never had been investigated on human muscle and since the paresis after application could be due to the blocking action of large amounts on the neuromuscular junction, it seemed of interest to investigate the effect of small amounts of acetylcholine on human muscle, and to study differences in threshold for acetylcholine in normal and pathological cases.

In normal subjects intra-arterial injection of acetylcholine into the brachial artery produces a motor response of short duration. The reaction usually occurs as a localized flexion of one or more fingers. It is succeeded by a vasomotor reaction (flushing and sweating), which disappears in the course of few minutes.

The threshold dose of acetylcholine to produce a motor response was found in 22 persons to 200—300 μg. In women the threshold is slightly lower than in men (maximum 250 μ g). in one case the threshold was 150 μg.

In contrast to what had been concluded from former experiments on myasthenic muscles a considerably higher threshold (400—800 μg) was found in 5 cases (4 females). After intramuscular injection of prostigmine the threshold approaches normal values.

As it is the case in anmial experiments, denervation in man is accompanied by a significant decrease in the acetylcholine threshold. Both in traumatic lesions of the peripheral nerves, in amyotrophic lateral sclerosis and poliomyelitis the threshold values are below 150 μg. The examination of genuine muscular disturbances revealed a remarkable increase in threshold (to 400—800 μg).

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VON EULER, H. (Stockholm): **Biochemical action of streptomycin.**

VON EULER, CURT; Stockholm: **Stimulation of the peripheral portion of the cut vagus nerve with constant currents.**

Experiments were carried out with cats, decerebrated or in chloralose anaesthesia. Polarizing electrodes were applied to the peripheral part of the cut vagus nerve in the neck and the blood pressure recorded. In addition the heart beat was followed by electrocardiography. To stimulation with polarizing currents of long duration this preparation responded with a marked r i s e of blood pressure after at latent period of some 10 sec. There was no change of heart rhythm. This effect was

still found after removal of the branches to the lung and section of the main stem just above the diaphragm. Atropine did not influence it. However, decapitation abolished the effect and so did removal of the stellate ganglion. The effect could not be referred to acceleratory fibres in the vagus.

VON EULER, U. S.; Stockholm: 1—**Noradrenaline and histamine in autonomic nerves.**

It has been discovered recently that mammalian postganglionic sympathetic fibres contain considerable amounts of a sympathomimetic ergone indistinguishable from noradrenaline (arterenol) in its biological actions (3.6). When extracts of splenic nerves from cattle were compared with laevo-noradrenaline (9) biologically and colorimetrically — using the author's iodine method — a very good quantitative agreement was obtained. In order to determine small amounts of adrenaline in the presence of noradrenaline in extracts, advantage can be taken of the observation that the latter substance has only about 1/30 of the activity of the former on the fowl's rectal caecum when the l-forms are compared. Since the effect on the blood pressure of the slightly ergotaminized and atropinized cat is about 4 times greater for l-noradrenaline than for l-adrenaline, these two biological tests allow the detection of even small amounts of adrenaline in a mixture of this substance and l-noradrenaline. It was found that less than 2 p.c. of the sympathomimetic activity of splenic nerve extracts was due to adrenaline, and approximately the rest to l-noradrenaline (6).

Apparently, the noradrenaline found in adrenergic nerves, corresponds to the sympathin E (excitatory) of C a n n o n and R o s e n b l u e t h. It is proposed that this ergone should be termed Sympathin N, since the term Sympathin E is inadequate in view of the fact that the active substance has weak but definitite inhibitory properties. Sympathin I may well be adrenaline, deriving from scattered groups of chromaffine cells present in almost all sympathetic nerves, and ganglia (5).

Extracts of sympathetic nerves of cattle contain large quantities of histamine (4). Thus the histamine content of the splenic nerves of cattle amounts to some 100 μg histamine dihydrochloride per g fresh nerve, extracted without its sheath. The highest figures were found in nerves containing postganglionic sympathetic fibres, though the sympathin N content bore no fixed relation to the amount of histamine.

This suggests that the histamine is bound to specific fibres within the autonomic nervous system. The optic nerve and

spinal roots contain small amounts only, as stated previously (8) (less than 15 μg/g), the vagus, chorda tympani, and short ciliary nerves are intermediate (20—30 μg/g), whereas the sympathetic chain, the vertebral and prevertebral ganglia, and the postganglionic fibres contain larger quantities. Some of the histamine present in excised fresh splenic nerves of cattle is released upon electrical stimulation of the nerve as shown by Euler and Åstrom.

It is inferred from the above observations that certain autonomic postganglionic fibres act by liberating histamine, i. e. serve as true histaminergic fibres.

The presence in autonomic nerves of three kinds of fibres with different ergone characteristics, viz. cholinergic, adrenergic, and histaminergic, suggest differences in other respects as well in these systems. Such have in fact been described for autonomic ganglia and their preganglionic fibres (1,2).

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v. EULER, U. S. and LILJESTRAND, G. (Stockholm). **Studies on the pulmonary arterial blood-pressure.**

It has been demonstrated by numerous workers that the systemic arterial blood-pressure is regulated through reflexes evoked by stimulation of baroreceptors and chemoreceptors as well as through direct action of the blood on the vasomotor centre. It seemed of interest to investigate whether similar influences are exercised on the pulmonary arterial blood-pressure.

Experiments were performed on cats under chloralose anaesthesia, a special cannula according to Mellin being inserted in the wall of the pulmonary artery. It was connected with a vertical narrow glass tube filled with Ringer solution and the pressure recorded with a piston recorder. In most cases the thorax of the animal was closed and spontaneous respiration restored.

Clamping of the common carotids elicited the usual rise in *the* systemic pressure, but had hardly any influence on the pulmonary arterial pressure. Occlusion of the left lung artery led to a rise of *about 20%*; moderate muscular work induced by electrical stimulation caused an increase of about 30% in the pumonary arterial pressure. Section of the vagi did not influence these results.

Breathing of pure oxygen lowered the pulmonary arterial pressure. Section of the vagi did not influence these results.

Breathing of pure oxygen lowered the pulmonary arterial pressure and oxygen-lack raised it without notably influencing the pressure in the left auricle. A mixture of carbon dioxide 6.5—20%) and oxygen raised the pressure slightly. Neither vagotomy nor exstirpation of the stellate ganglia had any influence on the effect produced by oxygen or oxygen want on the arterial blood-pressure. The same held true, as found recently by Logaras, for ergotaminisation or atropinisation of the animal.

It is concluded that alterations in the oxygen pressure in the alveoli have a direct influence on the pulmonary vessels, an increased pressure leading to a dilatation and a reduction to a contraction. By this mechanism an adequate distribution of the blood through the various parts of the lungs, according to the efficiency of aeration, is enabled. The results also seem to have some bearing on such questions as the therapeutic application of oxygen, its toxic effects, the influence of oxygen pressure on vital capacity, atelectasis, &c.

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FOLKOW, BJØRN and UVNÄS, BØRJE (Lund). **The chemical transmission of vasocontrictor impulses to the hind limbs of the cat.**

Sympatholytic drugs such as ergotamine and dibenamine reverse the vasoconstrictor action in the muscles of the hind limbs produced by adrenaline, and of stimulation of the abdominal sympathetics. In cross-circulation experiments we investigated the action of ergotamine and dibenamine on reflex vasoconstrictor responses in the hind limbs.

Cross-circulation was arranged between two cats in such way that all vascular connections between the upper and lower parts of the recipient cat were blocked and that the intact abdominal sympathetic chains constituted the only nervous connection between these parts. Vasoconstrictor discharges to the hind limbs were produced by reflex activation of vasoconstrictor centres. Under the influence of ergotamine or dibenamine the vasoconstrictor responses were reduced or annulled but never reversed to vasodilator responses. Since there is no indication that ergotamine or dibenamine interfere with the release of the adrenergic transmitter our experiments suggest that the transmitter at constrictor nerve endings is not adrenaline but a substance lacking vasodilator properties. From our observations nor-adrenaline fullfils this requirement. Under the influence of ergotamine or dibenamine it does not produce vasodilation.

The vasodilation produced in the hind legs under the influence of ergotamine or dibenamine by stimulating the sympathetics is adequately explained as due to the activation of vasodilator fibres. These fibres were found to be cholinergic.

FOLKOW, BJØRN and UVNÄS, BØRJE (Lund). **Sympathetic vasodilators in the cat and dog.**

The occurrence of vasodilatation on sympathetic stimulation in cats given dibenamine or ergotamine indicates the existence of sympathetic vasodilator fibres.

Bülbring and Burn reported that the sympathetic vasodilator fibres to the hind limbs of the dog are cholinergic. They believe that the corresponding vasodilator fibres in the cat are very few and adrenergic in nature. Our results indicate that the sympathetic vasodilator fibres to the hind limbs of the cat are cholinergic. The vasodilatation produced by sympathetic stimulation is enhanced by physostigmine and blocked by atropine. Furthermore stimulation of the sympathetic chain causes acetylcholine to appear in the perfusate from the hind limbs.

Sympathetic vasodilator fibres seem to be restricted to the muscular vessels. The vasodilatation in the hind limbs remained unaltered after the legs were skinned. Even after huge doses of sympatholytic drugs vasodilatation was never induced in the skin by sympathetic stimulation. Acetylcholine given intraarterially produced marked vasodilatation. Acetyl-

choline did not occur in the perfusate from the skin during sympathetic stimulation.

Vasodilator fibres to the coronary vessels run in the sympathetic outflow and are generally considered to be adrenergic. In experiments on dogs we found that minute amounts of acetylcholine given intraarterially induce pronounced were vasodilatation of the coronaries. When the sympathetics to the heart were stimulated acetylcholine appeared in the perfusate from the coronary vessels of the dog and cat. The observations indicate that the sympathetic vasodilators to the coronary vessels are cholinergic.

Our observations strongly favour the view that the sympathetic vasodilators both in the dog and cat are cholinergic. As previously reported by us the transmitter at vasoconstrictor nerve endings probably lacks vasodilator properties. The existence of adrenergic vasodilator fibres in these species therefore is unlikely.

FRANKENHAEUSER, B. (Oxford). **Sensory Impulses in Large Nerve Fibres from the Epiglottis of the Rabbit.**

The epiglottis of the rabbit is supplied with both large and small nerve fibres. The large fibres, however, are distributed to its posterior surface only. They terminate in complex endings of a very distinctive type.

The nature of the stimulus necessary to cause these endings to discharge has been investigated in the following way. The nerve supplying the epiglottis was exposed and cut about half a centimetre from the larynx. Action potentials were recorded in the usual way from electrodes placed on the nerve.

A continuous shower of large action potentials was recorded even when no respiratory air was allowed to impinge on the epiglottis. Air passing over the epiglottis caused an increase in the activity during expiration but a decrease during inspiration.

When the trachea was opened and a stream of air from the observer's lungs directed on to the posterior surface of the epiglottis the number of action potentials recorded increased and appeared to be similar to the increase seen during normal expiration.

There seems no doubt that there are sensory receptors in the epiglottis stimulated by respiratory air and therefore most probably concerned in the reflex regulation of respiration.

GABRIELI, ELEMÉR (Gothenburg). **Die Funktion der Magenschleimhaut. Experimentelle Untersuchungen mit radioaktivem Brom.**[1])

Die seit langem bekannte Tatsache, dass das im Blut zirkulierende Brom als Bromwasserstoff im Magensaft erscheint, und dass Brom in der Salzsäurebildung Chlor vollkommen substituieren kann, wurde neuestens von Davenport und Fischer (1940) bestätigt. Diese Resultate wurden von uns mit radioaktivem Brom nachuntersucht.

In der Herstellung von radioaktivem Brom mit Radium-Beryllium-Quelle (^{79}Br (n, γ) ^{80}Br und ^{81}Br (n, γ,) ^{82}Br wurde von Ingenieur Eric Berne für diesem Zweck eine verbesserte Herstellungs- und Abtrennmethodik ausgearbeitet. Anstelle des üblichen Szilárd-Chalmers Prozesses mit Äthylbromid, welcher nur ungefähr 30 % Separationsausbeute ergibt, wurde Natriumbromat in fester Form bestrahlt. Aus wässeriger Lösung wurde das Bromid-Ion nach Zusatz von wenig inaktivem Natriumbromid mit Silbernitrat gefällt, in der Wärme mit äquivalenter Menge Natriumjodid behandelt und so ging das radioaktive Brom in Form von Natriumbromid in Lösung.

In Chloralose-Urethan Narkose wurden Fisteln an Katzen-Magen angelegt, Cardia und Pylorus wurde unterbunden und das radioaktive Brom in physiologischer Lösung intravenös eingespritzt. 30—50 Minuten später wurde 1.0 mgr. Histamin subcutan gegeben und der Magensaft in jeder 15. Minute vollkommen abgesaugt. Mit üblicher Methodik wurde in jeder Portion die abgesonderte Menge, die freie- und gesamte Azidität, Gesamt-Chlor, und mit Zählrohr die Aktivität bestimmt.

In 14 Experimenten wurde beobachtet: 1./ dass ein Zusammenhang besteht zwischen der gefundenen Aktivität und der Salzsäurekoncentration. Wenn die Salzsäurekoncentration auf Histaminwirkung steigt, so sinkt gleichzeitig die auf 1.0 ml. gerechnete Impulszahl. Wenn die Salzsäurekoncentration später wieder abnimmt, die Aktivität bedeutend steigt. 2./ Trägt man das Verhältnis Br/HCl im entnommenen Magensaft als Funktion der A b s o n d e r u n g s g e s c h w i n d i g k e i t auf, so zeigt sich, dass bei schnellerer Absonderung /= Gasamte Azidität × Gesamte Menge × Minute $^{-1}$/ relativ mehr Salzsäure, als Brom ausgeschieden wird. Hieraus kann man schliessen, dass die Cl/Br Substitution bei der Sekretion des Magensaftes n i c h t vollkommen ist, sondern diese Relation eine Funktion der Sekretionsgeschwindigkeit ist.

[1]) Die Versuche wurden mit Hilfe von Wallenbergska Stiftelsens ausgeführt.

GERNANDT, BO (Stockholm). **Observations on single Fibres in the Mammalian Vestibular Nerve.**

With a needle micro-electrode it has been possible to isolate single fibres in the vestibular nerve of the cat. The response to rotational and caloric stimulation has been observed. The typical response to rotation is an increased frequency to rotation in either direction; a rare type of fibre, when spontaneously active, can be shown to stop discharging to rotation in either direction. These two types of fibre also respond to caloric stimulation as if the latter had been an adequate stimulus, hot or cold water accelerates the discharge that rotation would have inhibited. But in addition there is a third type of caloric effect which seems to be independent of the properties of the fibre. In those cases cold water completely blocks the discharge and hot water accelerates it. Compared with the transient specific effects of caloric stimulation the latter effect is of very long duration.

GLAVIND, J. and HARTMANN, S. (Copenhagen). **Heme-catalyzed reactions of organic peroxides.**

It is well known that heme can act as a peroxidase. The heme is able to catalyze the transfer of *1* atom of oxygen from hydrogen peroxide to a great variety of substances, such as pyrogallol, benzidine, guaiac resin, and leucomalachite green. Furthermore, it is known that also certain other peroxides, ethyl peroxide for instance, can be used as a source of oxygen instead of hydrogen peroxide in such reactions.

The most important organic peroxides in biology and food industry are the peroxides of highly unsaturated fatty acids. However, an attempt to study whether heme-containing compounds can catalyze a reaction between the peroxides of unsaturated fatty acids and oxidizable substances, has as far as we know only been made by F r e h d e n (Microchim. Acta *2*, 214 (1937)) who demonstrated the conversion by such peroxides of 2.7-diaminofluorene in the presence of hemin to blue meriquinoid oxidation products.

In the course of the work on peroxidized fatty acids in these laboratories it became desirable to develop a color reaction for these substances. It was found that under suitable conditions. peroxidized fats and fatty acids reacted in the presence of heme with a great number of oxidizable substances. Some examples of such reactions are the following.

If a few drops of a peroxidized oil are added to a solution of benzidine in alcohol, containing heme, only a very weak bluish color which soon after fades out, is seen, while under the same conditions the addition of a few drops of a weak solution of hydrogen peroxide produces a very strong blue color.

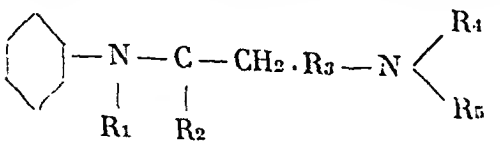
If, however, the reaction with benzidine is carried out in the presence of α-naphthol, as a result of a coupled oxidation a strong red color is obtained. In the same way a strong blue color of indophenol is obtained, by the use of *Nadi reagent*.

Very strong colors were found by the use of certain leuco-dyes. Leuco-malachite green was found especially suitable for the study of this reaction. The heme has its greatest effect when used in combination with pyridin. Heating, as well as the presence of a suitable amount of an acid, acetic acid, for instance, also accelerates the reaction. If water is used as a solvent, the reaction goes on quantitatively when hydrogen peroxide is used, but very slowly with organic peroxides. If, however, the water is substituted by an organic solvent, for instance acetone, the addition of organic peroxides gives a very strong reaction in the course of a few minutes. It can be observed, in this case, that when the reaction has started, an autocatalytic reaction takes place, resulting in the formation of a great amount of malachite green by decomposition of heme. However, if the reaction is carried out *in vaeno* under suitable conditions, a quantitative reaction takes place. Such a reaction can be used as a highly sensitive method for the quantitative determination of organic peroxides.

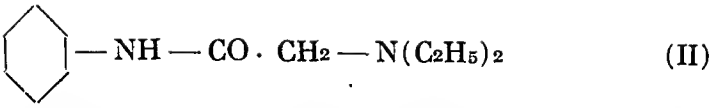
Under similar conditions as leuco-malachite green, also the leuco base of 2.6-dichlorophenolindophenol proved to be a sensitive reagent for the determination of organic peroxides.

GOLDBERG, LEONARD, Stockholm. **Anesthetic Properties of Alkylaminoacyl Anilides.**

The anesthetic action of compounds with the general formula (I) was observed by Erdtman and Löfgren (1937); specially alkylaminoacyl anilides (II) were found to be active.



(I)



(II)

Löfgren and coworkers (1946) have synthetized about seventy compounds, belonging to this series; extensive chemical and physico-chemical data are recently presented by Löfgren (1948). Fifteen of these compounds were submitted to elaborate pharmacological tests at the Pharmacological Laboratory, Karolinska Institute.

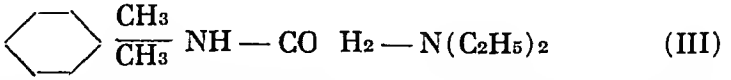
The following properties were investigated:

(a) t o x i c i t y by establishing convulsive (CD_{50}) and lethal dose (LD_{50}), on different animals;
(b) effect on b l o o d p r e s s u r e with and without the addition of adrenaline;
(c) a n e s t h e t i c properties: on the rabbits cornea, quantitatively recorded by means of a series of calibrated irritation hairs, and in dermal wheals on guinea-pig with and without adding adrenaline;
(d) possible i r r i t a n t action: on the rabbits eye, the rabibit's ear and intra- and subcutaneously in man.

The effects were compared to those of procaine (novocain), cocaine and tetracain (pantocain, decicain).

The new compounds are characterized by a high anesthetic activity, b o t h on the cye and in dermal wheals, a varying toxicity, and a depressant action on blood prcssure, being antagonized by the addition of adrenaline.

Absence of irritant action was noted for two of the compounds, viz. ω-diethyl-amino-2.6-dimethylacetanilide (LL 30, Xylocain /III/), and ω-diethyl-amino-2.4.6-trimethylacetanilide (LL 31).



(III)

Xylocain was submitted to a series of elaborate pharmacological tests (Goldberg 1947, 1948). The relative toxicity was found to be comparable to that of procain or lower, and decidedly lower than that of cocaine or tetracain. As a local anesthetic it was superior to procain for conduction anesthesia; it had a longer duration with a shorter latency time, a higher incidence of effect and a larger range of action, the effect being increased by the addition of adrenaline; for surface anesthesia it is comparable to tetracain. It is stable, not irritant and has a high safety coefficient. Xylocain has also been elimically tested (Bjørn and Huldt 1947), Bremer et al 1948, Gordh 1948).

A relation between chemical constitution and pharmacological effect of all the compounds investigated was established; the relation varied with the property studied: toxicity, anesthetic effect or irritant action: the results will be further discussed.

GRANADOS, HUMBERTO, GLAVIND, JOHS., HARTMANN, SVEND and DAM, HENRIK, Copenhagen. **A Histochemical Method for the Demonstration of Peroxides.**

The histopathological changes that take place in the adipose tissue of rats fed a vitamin E deficient diet, rich in highly unsaturated fatty acids have been studied to certain extent (Dam, H., and Mason, K. E., Fed. Proc. *4*: 153, 1945; Mason, K. E., Dam, H., and Granados H., Anat. Rec., *94*: 265, 1946; Granados, H., Mason, K. E., and Dam, H., Acta Path. et Microbiol. Scand. *24*: 86, 1947). However, in order to actually understand such changes it has proved indispensable to develop first a method for the demonstration of the very places where peroxidation occurs. Such a method should prove also important for the understanding of what the actual relation is between peroxidation and the acid-fast pigment (Dam, Mason, and Granados). In our previous paper "Heme-catalyzed reactions of organic peroxides" is described a reaction between peroxides of unsaturated fatty acids and the leuco-base of 2.6-dichlorophenolindophenol in the presence of heme. Applying the principle of this reaction, we have developed a method for the histochemical demonstration of peroxides, epsecially those of adipose tissue. In order to obtain sufficient velocity of the reaction, and to make possible its carrying out at ordinary temperatures, the staining was made in an alcohol-water mixture, in the presence of ample amounts of heme. So far we have studied this histochemical reaction in frozen sections of peroxidized bacon, and of adipose tissue of vitamin E deficient rats, with their respective controls. The sections are stained with the following solution, which should be prepared just before using it: 20 mg of hemin are dissolved in a mixture of 5 cc of pyridine and 10 cc of glacial acetic acid. This solution is mixed with 500 mg of the leuco-base of 2.6-dichlorophenolindophenol dissolved in 50 cc of absolute alcohol, and 120 cc of distilled water. The color begins to appear almost immediately. In this way is obtained a parallelism between the intensity of the red color and the content of peroxides. This fact was confirmed by staining sections of different pieces of tissue, the peroxide values of which had

been determined by King, Roschen, and Irwin's (Oil and Soap *10*: 105, 1933), and by Glavind and Hartman's methods. Permanent mounts are made using an aqueous mounting medium, such as Farrant's medium, and surrounding the cover glass with a cement, such as asphaltum.

GRANIT, RAGNAR (Stockholm). **Visual purple and the colour modulators.**

After a summary of the author's old work on the relationship between visual purple and the colour modulators some new results are presented showing that in the cat's retina the scotopic dominator, recorded from single fibres, often possesses humps in regions corresponding to the modulators. Sometimes, however, it actually reproduces the visual purple curve very well. In these new experiments the same element has been kept for a very long time under the micro-electrode so as to make it possible to take a large number of readings. The high sensitivity necessary for these humps to show above the contour of the extremely light-sensitive visual purple curve, particularly in the short wave-lengths, suggests that they represent transformations within the chromophore of visual purple of the type observed by Morton and his collaborators (Ball, Collins, Morton and Stubbs, Nature, 1948, 161, p. 424) by chemical methods.

HALD, JENS, JACOBSEN, ERIK and LARSEN, VALDEMAR. (Copenhagen). **Hypersensitivity against alcohol.**

Some substances and drugs will produce disagreeable symptoms after intake of ethylalcohol when such substances are absorbed by the organism, f.i. coprinus atramentarius (T. Fisher 1945) and after calcium cyanide (Koelsch 1914). As a third substance with similar properties we have found tetraethylthiuramdisulphide (Antabus). The present investigation is carried out with this substance, giving the same symptoms as seen after intake of the two afore mentioned substances.

After intake of 1.5 g Antabus a human subject feels no or only slight symptoms, but when alcohol is taken subsequently a characteristic effect is seen. Subjectively is felt heating in the face, palpitation and a slight dyspnoea, occasionally nausea. Objectively is seen a blushing in the face and

upper part of the chest accompanied by an increase in skin temperature. Moreover an increased pulse rate is seen and an increase in ventilation. The effect on respiration and circulation in human subjects is dealt with in the communication by Asmussen, Hald, Jacobsen and Jørgensen at this congress. The same effect is also seen in experiments with rabbits. The duration of the effect of a single dose of Antabus can be traced up to 8 days after the intake.

After previous treatment with Antabus no effect is seen on the elimination rate of alcohol. After intake of alcohol the concentration of acetaldehyde in blood is found to be 2—5 times that of untreated individuals after the same dose of alcohol. This is seen both in human subjects and in rabbits.

A possible explanation of the effect of Antabus after intake of alcohol lies in the formation of acetaldehyde, which is found to give a marked increasing effect on the ventilation and heart rate (Handowsky 1943). With continuous infusion of acetaldehyde in rabbits we have found that the same effect on ventilation occurs with concentrations of acetaldehyde in blood corresponding to those observed in rabbits treated with Antabus and alcohol.

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HALSE, KARL (Oslo). **Investigations on Hypomagnesemia in Dairy Cows.**

I have been partaking in a series of feeding experiments during which it has been possible to produce tetany and paresis in cows.

In the acute stage of the disease the cows showed hypomagnesemia and hypocalcemia, the average values for both minerals in blood serum agreeing very closely with those reported by Sjollema for cows with grass tetany.

In the experiments it was found that all diseased animals before the appearance of acute symptoms had developed a certain degree of chronic hypomagnesemia. The symptoms of tetany and paresis seemed to be induced by factors endangering the metabolic equilibrium of the animals, for instance underfeeding and the sudden onset of lactation after calving.

Generally, hypocalcemia did not appear in the hypomag-

nesemic animals until the date of the outbreak of the acute symptoms.

All affected cows had rations relatively poor in magnesium. But it has been possible to produce the disease in animals which had at least 7 g of magnesium a day in the preparatory period. It is therefore difficult to decide whether the disease is primarily caused by magnesium deficiency or by factors disturbing the normal magnesium metabolism of the cow.

Inanition experiments which were performed showed the importance of disturbing factors. Hypocalcemia and declines in serum magnesium resulted from inanition (lasting 1½ day) independent of previous intakes of Mg and previous levels of serum Mg. Any serious symptoms were, however, not observed in connection with these changes in serum minerals.

I have determined the activity of alkaline phosphomonoesterase in blood serum from some of the diseased animals regularly for more than a whole year. In serum from animals inclined to develop hypomagnesemia I found a positive correlation between serum magnesium and phosphatase activity. The phosphatase activity was in most cases at a minimum during tetany and paresis. But minima quite as low could be produced in the same cows under similar feeding conditions even when great amounts of Mg (about 30 g a day) were supplied. This occurred in spite of the fact that the magnesium additions had a marked effect upon serum Mg.

The observations seem to permit the conclusion that the pathological conditions which are met in grass tetany are not solely caused by magnesium deficiency.

HARTMANN, S. and GLAVIND, J. (Copenhagen). **A new sensitive method for the chemical determination of organic peroxides.**

In our communication "Heme-catalyzed reactions of organic peroxides" is reported that peroxides of unsaturated fatty acids can react with the leuco-base of 2.6-dichlorophenolindophenol in the presence of heme. In the course of this work it was found that the reaction between peroxides and the leucocompound alone took place with a considerable velocity, even though heme was not present. Since this reaction did not show a similar tendency to take an autocatalytic character as the heme-catalyzed reaction, it was attempted to use it as a basis for a chemical method for the determination of organic peroxides.

The determinations are carried out in the following way. From the oil, the peroxide value of which is to be determined, a suitable dilution is made by means of xylene. A small amount of the leuco-compound dissolved in acidified butanol is added, and the mixture is heated in a boiling water-bath for 10 minutes. After adjusting the volume to 5 ml, the intensity of the red color is read in a Beckman spectrophometer at 470 mμ. For the calculation of the result a curve for the extinction of 2.6-dichlorophenolindophenol in relation to the concentration of the compound is constructed. The concentration of the indophenol solution is determined by adding potassium iodine in acid solution and titration with thiosulfate. A content of 0.001 milliequivalent of peroxides corresponds to an extinction (log Io/I) of 0.56 in 5 ml of the final solution for 1 cm layer.

By this procedure peroxides values of less than 0.01 milli-equivalent per kg of oil can be easily determined. However, the reaction can be made still more sensitive by transferring the indophenol into its alkaline form, since the blue color of the dye in alkaline solution is much more intense than the red color in acid solution. The red color will change into blue by the addition of potassium palitate. In alkaline solution the leuco-compound has a great tendency to become oxidized by the oxygen of the air. Therefore, for most practical purposes, it will be convenient to use the light absorption of the red color. As a rule, this will be strong enough so that a conversion into the blue component is not necessary.

HITCHCOCK, M. W. S., KARVONEN, M. J. and PHILLIPSON, A. T. **The Effect of Insulin on the Acidity of the Abomasal Contents of Lambs.**

The abomasum in ruminants is the physiological equivalent of the stomach of other mammals. The abomasal juice contains HCl and appears to be under reflex control (Phillipson, 1936). Secretion of abomasal juice, however, occurs spontaneously after denervation (Popow & Kudrjavcev, 1930), contrarily to e.g. dog.

The effect of insulin hypoglycaemia on the acidity of the abomasal contents has been investigated in unanaesthetized lambs provided with an abomasal fistula. It turned out that the response in lambs is different to that previously described in other mammals. A distinct rise of the acidity after insulin was never observed. With a severe hypoglycaemia the acidity of the abomasal contents decreased markedly. The total

chloride concentration, however, varied within the normal *range showing* no consequent tendency either to a rise or to a *fall*. Altogether, 45 simultaneous determinations of the blood sugar and of the abomasal values were performed, and 26 of these were after the intravenous injection of insulin. A significant negative correlation (-0.66 ± 0.08) was found between the values for blood sugar and pH of the abomasal contents. A similar positive correlation (-0.55 ± 0.11) exists between the blood sugar and titratable acidity of the contents. With severe hypoglycaemia (blood sugar less than 10 mg-%) the average rise of the abomasal pH was more than 3 pH-units.

Cutting the vagi did not affect the qualitative response. The mechanism of the decrease in the acidity is therefore essentially peripheral. The decrease is best explained as being due to a suppression of the HCl-secretion. For establishing the validity of this conclusion, work using isolated pouches is in progress.

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JACOBSOHN, DORA (Lund). **The Action of the Corticotrophic Hormone on Mammary Gland Growth.**

The importance of the ovarian hormones for the mammary gland growth is well established. It is still open to discussion to what extent other hormones, chiefly pituitary hormones, have a stimulating effect. I have examined the action of the corticotrophic hormone on parabiotic rats under various conditions.

Two female littermate rats are sewn together side by side after opening their abdominal cavities. The resulting twins have a common abdominal cavity. Vascular and nervous connections of any importance are not established, an interchange of metabolites occurring through the pericapillar lymph spaces and the abdominal lymph system.

1) When a normal juvenile rat is joined to a castrated littermate the mammary gland shows an extensive proliferation of ducts and acini simultaneously with the ovarian hypertrophy.

2) On the other hand when a hypophysectomised animal is united together with a castrate the mammary gland remains unchanged in spite of persistent hypertrophic ovaries.

3) The junction of an adrenalectomised rat to a castrate results in a definite proliferation of mammary gland tissue, which, however, is less than that in the first series of experiments.

4) A combination of a hypophysectomised animal with an adrenalectomised castrate prevents the atrophy of the adrenals otherwise present in hypophysectomised animals and results in a slight degree of proliferation of the mammary gland.

These experiments indicate that there is some influence of the corticotrophic hormone on mammary gland growth via the adrenals. Compared with the influence of other pituitary factors the effect of the corticotrophic hormone is only slight.

JOUNI, JÄNNES (Helsinki). **On the Biosynthesis of Nicotinic Acid.**

Administration of sulfaguanidine to test persons during a period of from 5 to 6 days was found to reduce the secretion of nicotinic acid in the urine by, on an average, 30 per cent. By administration of succinylsulfathiazole an average reduction of 47 per cent was attained. This can be regarded as a result of the bactericidal action of sulfonamides on intestinal bacteria.

The nicotinic acid content of the blood, urine and faeces and the capacity of the faeces to synthesize nicotinic acid were compared. A vitamin-free culture medium was inoculated with faeces and the resultant amount of nicotinic acid was determined.

With persons suffering from chronic colitis the amount of nicotinic acid in the blood, urine and faeces was below normal, whereas that of the faeces culture was approximately equal in all persons.

In normal cases the average nicotinic acid content of the blood was 0.35 mg per cent, of the urine 0.18 mg per cent, and of 1 g of dried faeces 28 γ. The faeces contained on an average 25 per cent of dry substance, and the production of nicotinic acid from 1 g of faeces in 500 cc of nicotinic acid-free culture medium in 48 hours yielded on an average 80 γ.

The results obtained indiate that in conditions where intestinal peristalsis is increased, the amount of nicotinic acid synthesized by the intestinal bacteria is below normal. In the cultures, even the bacteria of these faeces are able to synthesize a normal amount of nicotinic acid.

The cyanbromide method has been employed in the past

for determination of the nicotinic acid content. The use of the Snell and Wright method of microbiological determination has now also been adopted and has proved very practicable.

JORPES, E., GARDELL, S., WERNER, BIRGITTA and ÅBERG, B. (Stockholm). **On the heparin monosulfuric acid. Its staining with fuchsinsulfurous acid after periodate oxidation.**

The main fraction of the easily soluble barium salts remaining in the mother liquor when protein free heparin from ox liver and ox lungs is precipitated as an insoluble barium salt has the composition of a heparin-monosulfuric acid. It contains equal parts of glucosamine, a uronic acid, acetic acid and sulfuric acid. It is dextrorotatory, the barium salt having a rotation of approximately + 50°.

The preparations are practically free of heparin di- and trisulfuric acids and have an anticoagulant activity of their own.

The anticoagulant activity of the barium salt varies between 10 and 16 provisional international heparin units per milligram.

The rate of hydrolysis at 100° C in 7.5 per cent sulfuric acid distinctly differentiates this polysaccharide from the chondroitin-sulfuric acid which hydrolyzes much faster in the beginning.

The yield of this fraction from the ox liver almost equals that of heparin, but from the lungs it makes up only a fifth.

All the heparin preparations are more or less inhomogeneous. This applies to the strongest ones with approximately three sulfate groups per each disaccharide unit as well as to the monosulfuric acid. It is questionable whether any chemically well defined heparin samples can be obtained.

The heparin monosulfuric acid gives a very strong color with fuchsin-sulfurous acid after periodate oxidation as does the hyaluronic acid. The stronger heparin preparations give, as is to be expected, no color, nor does the chondroitin-sulfuric acid.

When this reaction is applied to the tissue mast cells, their granules show a bright red color probably due to the monosulfuric acid of heparin or some sulfate free heparin precursor.

JOSEPHSON, BERTIL (Stockholm). **Studies on the excretory mechanism of the renal tubular epithelium.**

The transposition of intravenously injected diodrast (um-

bradil) from the plasma over the kidneys into the urine was studied mainly on rabbits but also on a few rats. In normal animals the diodrast concentration in the kidneys reached its maximum about 6 minutes after the injection and subsequently decreased roughly parallel to the plasma concentration. The diodrast concentration of the kidney parenchyma was also roughly parallel to the amount injected even if this reached lethal doses. This is in agreement with Röjel's observation that rabbits do not have any maximal tubular excretory capacity for fenol red. In animals with experimentally damaged kidneys, uranyl nephritis, hydronephrosis, pyonephrosis, nephroscirrhosis etc. the diodrast concentration of the kidneys reached values considerably lower than in normal animals but in these cases this maximal concentration remained nearly unchanged for at least one hour in spite of a decreasing plasma concentration. Here the "transpositor" was damaged or reduced.

In experiments in vitro it was found that kidney tissue suspended in a Ringer solution containing diodrast picked up more diodrast than liver tissue under corresponding circumstances.

In a spesial series of quite preliminary experiments one of the ureters was ligated in rabbits. 3—4 days after the operation the animals got diodrast intravenously and 7—20 hours after the injection the animals were sacrificed. After this time all diodrast that was not "fixed" had been washed out by the healthy kidney so that this, the plasma, and other organs were free from diodrast. Only the parenchyma of the ligated kidney showed a considerable diodrast-concentration for 10—20 hours after the injection. The hydronephrosis fluid did not contain any diodrast.

This remaining diodrast was considered as bound to the "transpositor" of the tubular epithelium only and this "transpositor" is now subject to further investigations.

Both in normal animals and in animals with one ureter ligated the capacity of the kidneys to take up diodrast was diminished if the animals had been treated with mercury diuretics. Urethan seemed to have a similar effect. Extracts from the posterior lobe of the hypofysis (hypadrin) seemed to help the kidney to increase the diodrast concentration of the kidneys that is to increase the possibility of the "transpositor" to take up substances for excretion.

JØRGENSEN, BARKER, C. (Copenhagen). **On the osmotic regulation in amphibians.**

When amphibians are kept starving in dilute salt solutions (e.g. tap water) they keep their body water and NaCl content at a constant level. The water taken up osmotically through the skin is eliminated at the same rate through the kidneys. NaCl lost in the urine and by diffusion through the skin is absorbed actively in equal amounts through the skin. — The effect of salt depletion and salt loading on the osmotic regulation has been investigated on the toad (Bufo vulgaris). Some experiments have been performed on Rana temporaria, too. Salt depletion was produced by keeping the animals in running distilled water. Salt loading was performed by given NaCl per os, by subcutaneous injection or by keeping the animals in 8 ‰ NaCl. After salt depletion or salt loading the animals were transfered to millimolar NaCl-solution. The total uptake of Na through the skin was determined by means of Na^{24} in the surrounding medium. Shortly the results were: When animals in need of salt were transfered to dilute salt solutions they increased the rate of active uptake of Na, whereas the loss of salt through kidney and skin was diminished. When salt loaded toads or frogs were transfered to water the rate of water absorption through the skin was greatly increased, often up to 3 times or more compared with normal values. The excessive water uptake decreased in the course of some few hours, and normal values were often found within 6 hours after the transfer to water. Together with the increase in water uptake a decrease in urine formation to values below normal was observed, resulting in an increased body weight. This effect of hypertonicity has a striking resemblance to the well known augmenting effect of posterior pituitary extracts on the body water of amphibians, the so called Brun effect. During the increased permeability of the skin to water the loss of salt is increased, too. But by far the greater amount (more than 90 %) of the surplus salt was found to be excreted through the kidneys.

JØRGENSEN, GUNNAR (Copenhagen). **Venous Pressure During Work.**

The purpose of this investigations is to correlate v.p. during varying degrees of work, and put it into relations to other circulatory functions. The experiments were made in the morning after a resting period in the horizontal position

of 45 minutes and 15 minutes on Krogh's bicycle ergometer, sitting in a low reclining chair. The O_2-intake and the a.-v-O_2-diff. were determined by means of the Douglas bag method and the acetylene method according to Grollman. After 10 minutes the v.p. was determined directly in a cubital vein. During continuous determination of the v.p. the work was started and carried on for 20—30 minutes. Before the work was finished, the cardiac output was again determined. Pulse rate and arterial pressure were continually determined.

The experiments show a gradual rise in v.p. during the first 7—15 minutes of the work to a nearly constant value that is maintained during the rest of the experiment. In a few cases a slight fall is found at the beginning, followed by the gradual rise. A curve representing the stroke volumes at different steady states of v.p. is of a hyperbolic shape, rapidly growing more horizontal with increasing v.p. This curve is considered as a pressure-volume curve of the heart, and is compared to pressure-volume curves and length-tension diagrams of isolated hearts and isolated heart muscle bundles. If the curve is drawn logarithmically, it is seen, that the steady state values during work are all lying on a straight line, while the resting values (with a large scattering in v.p.) are lying beneath this line. It is discussed whether the lowered meanintrathoracic pressure can be the reason.

Since cardiac output, pulse rate, arterial pressure and ventilation at the beginning of work reach steady state values in 2—3 minutes, the gradual rise in v.p. is remarkable. On that account v.p. was determined with the person at rest in a room, which in 30 minutes could be heated from 25° to 36° C., in order to determine if an increased blood flow through the skin vessels can rise the v.p. No alterations were found in v.p.

In other experiments v.p. was determined at the transition from rest to work, from work to rest and from one work intensity to another. In all cases but one it took at least 5 minutes, before v.p. had adjusted itself to a new steady value. It is assumed that alterations must take place in the heart itself.

In some experiments, which are not yet completed, chymografic X-rays of the heart are taken at rest and at different moments during and after work. It appears, that the cross diameter of the heart is a little smaller during work than during rest, and it is always diminished after work for several minutes. This must mean, that the residual blood of the heart is diminished after work and perhaps also during work.

When work starts the heart is partly emptied of residual

blood and is filled at a comparatively lower pressure than during rest. In the following minutes alterations occur in the heart muscle, so that the filling pressure must rise to ensure an adequate filling.

KAHLSON, G. (Lund): **Trends in basic medical research.**

KNAPPEIS, GUSTAV G. (Copenhagen). **The Influence of Temperature on Birefringence of the Muscle Fibre and Actomyosin Thread.**

The birefringence of living cross striated muscle fibres of the frog at equilibrium length is $2.0 \times 10^{-3} \pm 0.05 \times 10^{-3}$ (20° C.). In freshly prepared actomyosin threads it amounts to approximately 0.04×10^{-3} (20° C.). In the isolated muscle fibre it is only slightly influenced by stretch, i. e. 0.02×10^{-3} per 10 per cent extension. In actomyosin threads which at equilibrium length are much less orientated than muscle the increase in birefringence due to stretch is about 5 times higher. Drying of the actomyosin threads previous to renewed immersion (0.9 per cent sodium chloride) likewise increases birefringence from approximately 0.04×10^{-3} to 1.25×10^{-3} (20° C.). Both extension and drying probably cause an increase in orientation.

The birefringence of muscle fibres and actomyosin threads at equilibrium length is reduced with increasing temperature. In the average the decrease amounts to 0.5 per cent/° C. in muscle and 0.7 per cent/° C. in actomyosin threads. The mechanical tension in the resting muscle fibre increases with increasing temperature by approximately 0.15 per cent/° C. Over the range of temperature investigated (0°—30° C.) birefringence varies in both muscle fibre and actomyosin thread linearly with the temperature and the decrease in birefringence is more than corresponding to a reverse proportionality with the absolute temperature.

In actomyosin threads the temperature dependence of the birefringence decreases with increasing stretch corresponding to the reduced influence of thermal agitation at higher extension. In the muscle fibre the reverse is the case, the temperature dependence up to a stretch of 50 per cent increases from 0.5 per cent/° C. at equilibrium length to 0.7 per cent/° C. A further extension does not produce measurable changes in the temperature dependence.

The different influence of temperature on birefringence in actomyosin threads and muscle fibres indicates the pres-

ence of changes in minute structural elements in the muscle fibre which exceed the orientating effect of thermal agitation. This finding is in agreement with the conclusions drawn from investigations of mechanical properties in this laboratory.

KOEFOED, HENNING (Copenhagen). **A preliminary report on the histology of partially corticectomised rat-kidneys.**

A preliminary report on investigations of the behavior of the kidneys of white rats after partial, unilateral corticectomy is given here. The investigations are carried out partly by the maceration method and partly by the usual histological method. On the partially corticectomised kidneys it is tried to ascertain whether the glomerules and tubules in the cut nephrons atrophy, regenerate or function further.

The single parts of the undamaged nephrons from the operated kidneys are measured and compared with the corresponding parts from the kidneys of normal animals in order to state if hypertrophy occurs.

The undamaged kidneys of the unilaterally corticectomised animals are examined for possible hypertrophy and, in addition the nephrons are measured and compared with those of normal animals.

The changes that occurred in the kidneys in the course of about three months after the corticectomy was made are demonstrated by means of photographic exposures, partly of the macerated preparations and partly of the usual histological preparations.

LANGFELDT, EINAR (Oslo): **The formation of hydrochloric acid.**

The action of bicarbonate on the formation of hydrochloric acid found by earlier workers in dogs, is confirmed on rats.

A theory for the accumulation of hydrogen ions in the parietal cells as a process depending on an autocatalytic CO_2-cycle, and the elimination of hydrogen ions in the canaliculi as a Donnan-effect depending on the presence in the cells of positively charged protein ions is proposed.

LARSEN, VALDEMAR (Copenhagen). **The effect of Adrenaline and Ephedrine on the coronary vessels.**

In an earlier paper it has been shown that adrenaline produces dilatation of the coronary vessels in the isolated heart of the rabbit, whereas the related ephedrine gives constriction. This result does not agree with G a d d u m's theory

according to which the effect of ephedrine is to be regarded as a potentiated effect of adrenaline because ephedrine should inhibit the breaking down of adrenaline. The present investigations demonstrate that adrenaline and ephedrine employed simultaneously constantly behave as antagonists and that the effect is a simple additive one. This result would be in accordance with G a d d u m's theory if it is assumed that not adrenaline but a metabolite of adrenaline dilates the coronary vessels, whereas adrenaline itself produces constriction. However, investigations about this do not confirm this probability: When adrenaline was incubated with minced heart muscle plus blood at 37° C. and at pH = 7.3 until ⅔ of the adrenaline was broken down, the mixture showed the same dilating effect on the coronary vessels as a solution of pure adrenaline with the same concentration. This would indicate that the dilating effect of adrenaline is attached to adrenaline itself and not to a metabolite of the last mentioned.

Thus the view must be maintained that adrenaline and ephedrine in the isolated heart of the rabbit have an antagonistic effect on the coronary vessels and that it is adrenaline itself which has the dilating effect on the vessels.

LAURENT, B. S. L. (Helsinki). **On the Amount and Nature *of Polysaccharides in Urine Proteins, as Compared* with Serum Proteins.**

The studies reported in literature on urine proteins in renal diseases have indicated that these proteins probably are identical with serum proteins in respect to the amino acid content and behavior in electrophoresis tests. In comparison to this, our knowledge of their sugar content is very meager. It was the purpose of the present work to endeavor to shed light upon the latter feature by means of simultaneous analyses of the polysaccharide groups of urine and serum proteins.

Fractionation in albumin and globulin is carried out with ammonium sulphate, which is then eliminated by dialysis. Coagulation of the protein is produced in a water-bath with acetate buffer, after which it is washed, dried and dissolved in diluted alkali. The carbazole method in the form developed by Gurin and Hood was used at first for the sugar determinations, but as it did not prove practicable for differentiation of galactose and mannose the orcinol method advocated by Sørensen and Haugaard was adopted. A modified tryptophane method was also tried out.

LINDE, SVEN (Uppsala). **Further Experiments on the Primary Acidity of the Gastric Juice.**

The technique employed is described by T. Teorell, K. J. Öbrink and the author.[1]) The experiments were performed on stomach pouch dogs. Gastric secretion was induced by continuous, intravenous histamine, pilocarpine or insulin injections. To prevent "back diffusion" of H-ions glycocol was used as buffer substance in the pouch. Glycocol is poorly diffusible through the stomach mucosa und unlike many dyes is not adsorbed by the mucus. The glycocol was utilized not only as a buffer substance, but also as a "dilution indicator", giving a measure of the secreted volume by a simple calculation. The volume increments directly observed in a measuring cylinder during the glycocol instillation agreed very closely with those indirectly calculated. The H-ions added to the buffer by gastric juice were titrated from these data and the concentration of H-ions in the pure gastric juice was calculated. The results of pilocarpine and insulin experiments are compared with those obtained by histamine and discussed with regard to the current views of a constant primary acidity.

LUNDBERG, ANDERS (Stockholm). **Potassium and the thermal sensitivity of mammalian nerves.**

The effect of local temperature changes, set up by a thermode, has been studied on isolated motor roots (cat) in Krebs solution with membrane potential, negative after potential and spike height as index. In response to a modest increase of the potassium concentration of the medium the negative afterpotential disappears; the membrane potential and the spike potential shift their maxima to a higher temperature range than in untreated nerve. Similarly the conduction block in response to cooling shifts to a higher temperature range. Opposite changes are obtained when the nerve is treated with potassium-free Krebs solution. The maximum of membrane potential and action potential shift to a lower temperature range than in untreated nerve and, in order to block conduction, it is necessary to cool the nerve more than had been necessary for an untreated nerve.

[1]) Acta Physiol. Scand. *14*, 220, 1947.

LUNDERVOLD, ARNE (Oslo). **Electromyography. A technical defect.**

In Electromyography with more simultaneous earthings a leakage of activity may take place from one electrode to the other. This may occur when there is a connection between the various channels (magnifiers) of the electromyograph and provided there is electric contact between the electrodes; besides it depends on the construction of the amplifier. The first condition will exist almost always, as most electromygraphs are connected with earth in one way or other. The second condition — electric contact between the electrodes — will always exist, when one is deriving from one and the same person.

The possibility of eliminating this leakage will therefore entirely depend on the construction of the amplifier. When using an ordinary amplifier with the central conductor of the electrode connected direct to the grid of the first tube, while the canulla itself is connected to earth, the leakage will be very great — up to 100 %. A push-pull amplifier will reduce the leakage considerably, and the use of a differential amplifier will reduce it still more.

The above-mentioned leakage has been found to complicate the interpretation of the electromyogram, and it has also been shown by demonstration how it can result in the formation of synchronic units.

LUNDQUIST, FRANK (Copenhagen). **The Viscosimetric determination of Hyaluronidase.**

Of the many methods which have been used for the estimation of hyaluronidase activity, those using the decrease in substrate viscosity appear to be best suited for quantitative measurement. Evaluation of the time necessary for reduction of the relative specific viscosity to half the initial value under specified conditions of pH, temperature, buffer- and substrate-concentration, etc. seems to be a principle in extensive use, though other modifications of the viscosimetric method have been published. Certain practical and theoretical objections may, however, be raised against this method:

1) The time of mixing enzyme and substrate must be well defined — which is not easy to ascertain because the viscous substrate has little tendency to mix with the enzyme.
2) The initial viscosity must be known, requiring either

extrapolation — which gives uncertain results — or a special measurement with inactivated enzyme.

3) Each viscosity measurement requires considerable time, during which the quantity measured decreases, thus introducing a new source of error.
4) The use of the "half-viscosity-time" implies that the enzymatic reaction is monomolecular, — but this is not the case under the conditions used.

When a suitable substrate concentration is used, the first part of the reaction (up to about 20 per cent reduction of the viscosity) follows the monomolecular scheme. When the logarithm of the viscosity is plotted against time a straight line is obtained. The slope of this line (the reaction constant) is a measure of the enzyme activity. When the substrate concentration is kept within certain limits (i. e. when the flowtime is proportional to the substrate concentration) the logarithm of the flowtime may be used directly instead of the viscosity.

When this principle is used, most of the drawbacks of the viscosimetric method are avoided. If the preparation of hyaluronic acid used as a substrate is sufficiently well defined, the reaction constant furnishes a means of defining a (badly needed) international unit of hyaluronidase. The possibility of making preparations of hyaluronic acid with well defined physical properties is discussed.

LUNDSGAARD, EINAR (Copenhagen). **The glyconeogenesis in the isolated liver.**

The carbohydrate and urea formation in the isolated cat liver has been determined under varying conditions. Results: D/N-quotient in the order of 4 to 5. Absolute glucose output 1—2 mg pr. minute. As the glucose utilization in the extrahepatic tissues of a cat at normal or slightly increased blood sugar level is about 7 mg pr. minute the observed glucose output from the liver should not suffice to maintain normal blood sugar level.

As we have good reasons to assume that the metabolism of the liver is influenced from the periferal tissues via the blood a series of experiments were carried out in the following way. A cat was exviscerated or hepatectomized. The blood sugar was kept as far as possible constant by continuous intravenous injection of glucose permitting a calculation of the glucose utilization in the preparation. A liver was

isolated and run with artificial perfusion for 35—50 minutes. The glucose formation in the liver was determined. Then the isolated liver was connected with the exviscerated or hepatectomized preparation. Venous outflow from the liver to jugular vein. Outflow from carotic artery to a small reservoir to which the pump perfusing the liver is shifted. Determinations of oxygen, carbon dioxide, glucose and lactic acid in blood going to and from the liver. Results: Oxygen consumption increases 100 % or more. No characteristic changes in R.Q. Glucose utilization in the extrahepatic tissues unchanged. Glucose output from the liver increases about four times. This increase is due to conversion of lactic acid into glucose and not to a glyconeogenesis proper. The marked increase in oxygen consumption is due to factors other than the increased lactic acid concentration in the blood going to the liver. The cause of the increase in oxygen consumption is under investigation.

MOLLAND, JACOB and BONNYCASTLE DESMOND B. **Studies in Pain Threshold. A Study of Aspirin's Potentiating Action on the Analgetic Activity of Codeine.**

In this study we have used radiant heat as the painful stimulus, employing a modification of the D'Amour-Smith apparatus as the source of radiant heat. Rats have been used as the test animals. The tails of the animals were exposed to the heat and the reaction time taken as that time when the tail is removed from the beam of light. With this type of design for studying the effects of drugs upon pain threshold certain considerations are of great importance: 1) The animals must be trained before use. 2) In order to avoid injury to the area of stimulation, which would impair the animal's usefulness, it is necessary to establish, daily, a period of maximal allowable stimulation (cut off time). 3) There should be an adequate statistical design employed in carrying out this type of experimental work.

In our experiments we have used a statistical design which consisted of two 9×9 Latin Squares, thus ensuring that every day of the assay procedure every animal received a drug, and that every day of the assay every dose of every drug was given. Since we have used a cut off time, our results only give an estimate of the incidence and duration of analgesia, not of the depth or absolute elevation of the pain threshold.

Acetyl salicylic acid and codeine sulphate were given intraperitoneally separately and in combination, in graded doses. in order to examine the potentiating action of the acetyl salicylic acid upon the analgetic activity of codeine.

The results obtained indicate that a combination of aspirin and codeine given intraperitoneally to the rat resulted in a greater incidence and a longer duration of analgesia.

MALM, O. J. (Oslo). **Electrolytical kat-ion analyses with a permanent aluminium-oxide-filter.**

MUNCK, KJARTAN (Copenhagen). **Role of Inositol in distribution of fat in the Organism.**

The first step in the investigation of the lipotropic action of inositol here reported was to try, with our strain of rats, to reproduce the recent works of McHenry, who produced a fatty liver, resistent to dietary choline.

In a period of 3 weeks a number of male albinorats weighing 100—110 g were fed a vitamin-B-free diet, poor in choline, containing sucrose 84 %, casein 10 %, salt 4 % and agar 2 %. Water ad libitum + A- and D- vitamin.

After this depletion period 5 groups of animals, 10 in each, were fed for 8 days the same diet as above, and given a daily supplement of B vitamins by subcutaneous injections, and, by mouth, the liver fraction, which according to McHenry produces a fatty liver resistent to choline. For further supplement, see table 1. The animals were killed, the livers removed, weighed and analysed for fat. The fatty liver, thus produced, was resistent to choline but not to insotol as seen from table 1.

Table 1.

Group	I	II	III
Supplement	None	0.5 % Choline	0.5 % Inositol
Total fat per cent of wet liver	18.7 $\pm$ 1.3	18.4 $\pm$ 1.8	7.7 $\pm$ 0.7

In a later experiment the carcasses (without liver and digestive tract) were analysed for fat to see, if it was possible to find an increase in extrahepatic fat in animals fed inositol. The results of these investigations showed a difference in the amount of fat in the animals without (9,4 % $\pm$ 0.5) and with

dietary inositol (12.0 % ± 1.0). Under certain circumstances one will get an incomplete impression of the role of inositol in distribution of fat in the organism if only the liver fat is taken into consideration.

MURHU, KASTEHELMI (Helsinki). **On the presence of Ehrlichs mast cells in frog in different phases of development.**

About 200 frog larvae in different developmental stages from ova to fullgrown frogs were examined as to the presence of Ehrlichs mast cells in different organs.

Ehrlichs mast cells appear in frog during metamorphosis simultaneously with the beginning of breathing with lungs.

During and immediately after thyroxine treatment a great number of mast cells appears in the spleen of the larvae. After a few days the number of mast cells in the spleen decreases and a simultaneous increase in the alimentary tract is stated. This phenomenon may be interpreted by presuming either that the mast cells are formed in the spleen and wander from it into other organs or that the thyroxine strongly stimulates the secretory activity of the mast cells in the spleen (appearence of metachromatic granules) followed later on by the disappearence of the granules. In absence of granules the cells are no more discernible.

The sequence of the appearence of mast cells in the spleen and alimentary tract is dependent upon the rapidity of metamorphosis. In individuals with arrested development (semineothenic frogs) the appearence of mast cells corresponds to the beginning of breathing with lungs as it does in those with speeded metamorphosis (in larvae kept in room temperature).

Between the mast cells of normal adult summer and winterfrog there is but little difference. The number of mast cells and the number of granules contained in them is slightly increased in the tongue, oesophagus and ventriculus of the summerfrog, quite in accordance with the increased vital functions.

NICOLAYSEN, RAGNAR (Oslo). **Studies upon the absorption of calcium in rats.**

The absorption has been studied extensively both in intact rats and from isolated loops of the small intestine. A 24 hours period gives more consistent results than a 5 hours period in isolated loops experiment. Within the same group

of rats no correlation was found between the absorption from the intact alimentary canal and from the isolated loop.
However clear cut differences is observed between old and young rats, the latter type aborbing Ca much quicker than the old ones. The authors old experiments with vitamin D has been repeated with salts as Ca chloride, gluconate, acetate and laevulinate. In altogether about 20 series of each ten rats, half of which received pretreatment with vitamin D others not it was observed consistently that rats given vitamin D absorb much more Ca than rats on a vitamin D free diet. Gluconate is absorbed quicker from isolated loops than the other salts, but not in intact rats, where the same results are obtained.

NICOLAYSEN, R. and NJAA, L. R. (Oslo). **Studies upon the effect of phytic acid on the absorption of calcium in rats.**

7 months old rats were used. They lived on a phytic acid rich ration from weaning until the studies started.

The calcium absorption was then studied for 7 months in which they were given alternating in 2 weeks periods wheat flour of 95 % extraction, 60 % extraction and the same 95 % extraction flour, in which the phytic acid had been digested prior to experiment. In the first 5 months of this series a steady effect of phytic acid on the absorption of calcium was observed. The percentage absorption of the standard test dose of calcium (0,25 % in the diet) was slowly reduced throughout the 7 months. In the last month the calcium absorption was only about 20 % and no clear cut difference was observed between periods on white as compared to periods on brown flour or «digested» brown flour.

(The basal diet consisted of 80 % flour, 9,5 % arachis oil, 5 % eggalbumen, 3 % dried brewers yeast, NaCl 2 %, $CaCO_3$ 0.25 %. The rats received 400 I. U. vitamin D weekly and 20 I. U. vitamin A daily.)

NÆSS, KNUT (Oslo). **The irritability of the motor nerve during ether anesthesia.**

The irritability of the motor nerve during clinical ether anesthesia has been examined as a part of the investigation of the effect of ether anesthesia on the peripheral motor system. The experiments have been carried out on the isciatic nerve of rabbits.

This matter has been investigated many times in the past with greatly varying results. Even the most recent investigations in this field have given divergent results.

It is pointed out that one, when investigating the effect of ether on the peripheral motor apparatus, should plan and carry out the experiments in such a manner as to examine so far as possible each part of the peripheral motor system separately.

The experiments have been conducted in vivo during an ordinary clinical ether anesthesia. In such experiments, special demands are made upon the electrodes which are used. It has, therefore, been necessary to develop a new type of electrodes.

The significance of the nature of the stimulus and a proper criterion for the threshold stimulus is being discussed.

Partly, investigations have been made of strength-duration curves on the various stages of anesthesia, and partly continuous tests have been made in order to determine the rheobase values. The stage of anesthesia is determined by analysis of the ether concentration in mixed venous blood.

The investigations have given the following results:

The irritability of the motor nerve decreases during anesthesia, and when respiration ceases it is approximately 50 % below normal.

The effect on the irritability is not proportional to the ether concentration, but it increases rapidly during the last stages of the surgical anesthesia.

Both strength-duration curves and direct tests show that the chronaxie decreases when the rheobase value increases. During an anesthesia which results in cessation of respiration, the chronaxie decreases from approximately 115 to 85 microseconds.

An increase in the irritability at the beginning of anesthesia has been demonstrated repeatedly, and one might, therefore, with a reasonable degree of certainty assume that the ether during this period can have a stimulating effect. On the other hand, a corresponding increase in the irritability after the end of anesthesia has never been recorded, even though the ether concentration in mixed venous blood has returned to its original value.

The investigations also seem to point to the fact that increased irritability of the nerve takes place only at a relatively rapid supply of ether, and that ether has a stimulating effect only in small concentrations while penetrating into the nerve cells.

This result is compared to similar theories regarding the stimulating effect of alcohol.

OBEL, N. J. and SCHMITERLÖW, C. G. (Stockholm). **The action of histamine and other drugs on the bronchial tone in horses suffering from alveolar emphysema («heaves»).**

Bronchial tone in horses has been studied by recording intrathoracic pressure changes.

Horses suffering from heaves show marked positive expiratory pressure and a lower negative inspiratory pressure than do normal horses. The intravenous injection of adrenaline in such horses almost instantaneously reduces the intrathoracic expiratory pressure, causing a dilatation of the air passages, the respiratory curve becoming fairly normal. Atropine, scopolamine and methylscopolamine nitrate exert the same effect, the duration of their action being considerably longer.

Horses suffering from heaves show a high sensitivity towards histamine which causes a very forced respiration obviously due to a marked bronchoconstriction; this is brought about even when the bronchi have been relaxed by atropine or when the symptoms of the disease have disappeared. Normal horses are more resistant to histamine.

The high histamine susceptibility in heaves may suggest a possible allergic factor in this disease, which in many respects resembles bronchial asthma in man.

PALMER, H. (Oslo). **Sex Hormones and Serum Iron.** (A preliminary report). It is found that the serum iron level in normal rabbits decreases by parenteral injections of sex hormones.

PEKKARINEN, A. I. (Helsinki). **On the Chemical Determination of Adrenaline by the Fluorescence Method.**

For determination of low adrenaline contents in biological material by chemical procedures, a method has been developed which is based upon the adsorption of adrenaline by aluminium hydroxide at pH 8—8.5 and upon utilization of the fluorescence reaction characteristic of adrenaline. The adrenaline is dialyzed for a period of from 5 to 6 hours from sodium citrate blood into a water solution containing aluminium hydroxide. The adrenaline in the dialyzing fluid becomes equalized to a given level with the adrenaline in the blood, and the pH of the dialyzing fluid, by the action of the ions dialyzed from the blood solution, become optimal for adsorption. By dissolving the aluminium hydroxide precipitate into alkali, determination of the adrenaline can still be made from the blood in a concentration of 0.5—1 g % by means

of the fluorescence reaction brought about by the alkali. Eosine solutions are used as standards of comparison.

Adrenaline still gives a fluorescence reaction in a slightly oxidized condition; it does not lose this property until at a later stage. The intensity and duration of the fluorescence reaction of the adrenaline caused by alkali are influenced by the reaction and temperature of the solution and by oxidizing and reducing substances. The oxidizing substances accelerate the reaction by catalyzation. By addition of greater amounts of oxidizing substances the reaction may become so rapid that the fluorescence caused in the adrenaline solution by alkali is not discernible. Reducing substances, on the other hand, retard the fluorescence reaction and ultimately inhibit completely the rapid oxidation of the adrenaline so that no fluorescence of the adrenaline solution occurs upon addition of the alkali.

It has not been possible to indicate adrenaline in peripheral venous blood. Clearly demonstrable amounts of adrenaline have been found only in vena suprarenalis blood and, if the secretion of adrenaline has been increased by insulin or morphine, also in vena cava blood.

Adrenaline disappears very rapidly from the blood stream. Injections even of large amounts of adrenaline into the blood stream of test animals are quickly adsorbed into the tissues apparently in the region of the capillary system, i.e. from the blood stream of the rabbit in 5, dog in 10, calf in 15, and cow in from 20 to 35 minutes. Even if, prior to the injection of adrenaline, the blood circulation of the liver, kidneys and intestines is closed off, the rate of disappearance of adrenaline will remain unchanged. Vitamin C and ephedrine are likewise unable to inhibit the rapid disappearance of the adrenaline from the blood stream.

In the tissues, the fluorescence of adrenaline is inhibited by reducing substances, in particular by vitamin C. Adrenaline added to minced tissue prepared from the liver, kidneys, brain, pancreas, thyroid or spleen will not fluorescence after dialysis upon addition of alkali; however, fluorescence can be produced by oxidation of the reducing substances by iodine. Adrenaline added to minced muscle, also of heart muscle, can after dialysis be brought out without oxidation nearly as well as from the blood.

RATJEN, ERLING (Aarhus). **The permeability of human red blood corpuscles after physical treatments.**

Some in vitro experiments including heat treatment and irradiation of human blood with X-rays, gamma rays and alfa particles are recorded.

The interchange of Na+, K+ and some "anelectrolytes" (glucose, malonamide, ammunium acetate, thiourea, hexamethylenetetramine) between the surrounding medium (serum, salt-solutions) and corpuscles is determined partly by chemical analyses and partly by volumetric methods (photoelectrically (Ø r s k o v) and by hematocrite). Further, the transformation in the shape, in the osmotic resistance and the conditions of hemolysis are studied.

By heating blood or a suspension at 50° or beyond this temperature — as other investigators have shown—corpuscles freshly drawn will brake into numerous spheres. This is independent of the osmotic concentration of the suspension if pH > 4, and there is no immediate associating hemolysis. The osmotic and physical resistance is lowered. The permeability for all investigated substances is enhanced.

After irradiation the permeability to kat-ions is immediately and considerably increased. The effect caused by radiations is delayed and less pronounced when "anelectrolytes" are test substances. The process in sequence to irradiation has a negative temperature coefficient. The effect is due to the action upon the erythrocyte membrane.

The dependence on the dose and on the type of the radiation forms a material fitted for testing the applicability of the target theory on the radiation effect.

SAND, HARALD (Oslo). **Carbonic Anhydrase in the salivary glands.**

The carbonic acid content of saliva has been investigated. It has been found that the concentration of bicarbonate in saliva is greatly increased when the secretion is activated, and may even rise considerably above the concentration in the blood. The bicarbonate of the blood therefore is probably not the only source, and it has been assumed that at least one part of the bicarbonate in saliva, is derived from the CO_2 produced in the glands during the secretion process.

The presence of the enzyme carbonic anhydrase in the salivary glands both in man and in the ox has been demonstrated. This enzyme catalyzes the hydration of CO_2. The CO_2 produced in the glands is therefore imideately hydrated to carbonic acid, which subsequently dissociates in hydrogen

ions and bicarbonate ions. Thus the increase in bicarbonate in saliva with increasing secretion is explained.

The pH and the buffer capacity of saliva is chiefly determined by its bicarbonate content. The carbonic anhydrase in the salivary glands therefore greatly influences the properties of saliva.

SCHIØLER, PETER (Copenhagen). **On normal and provoked blood alloxan.**

With the air of the method for determination of alloxan — developed by Archibald — it has been shown that oral ingestion of glucose or its normal metabolites — with the exception of malic acid — results in a marked increase of blood alloxan.

The blood alloxan determined after ingestion of 1 g/kg body wt. of pure glucose had a level of about 1.0 mg %. The intraperitoneal injection of responding amounts of alloxan — 20 mg/kg body wt. — resulted in hypoglycemia.

An attempt to produce hyperglycemia and glucosuria with mesoxalic and tartronic acids failed.

It is suggested that carbohydrate has a normal function in alloxan metabolism. Alloxan cannot be formed by mesoxalic or tartronic acids even in toxic amounts. Tartronic and mesoxalic acids show high toxicity.

The second part of the work suggests a stimulating effect of alloxan on insulin production, which is to be proved when the quantitative determination of insulin is possible by non-biological methods.

SEYFFARTH, HENRIK (Oslo). **The grasping phenomena in patients with cerebral lesion and in normal persons.**

In City Hospital, Boston, U.S.A., D. Denny Brown and myself did a clinical analysis of the involuntary grasping phenomena in 88 patients with cerebral lesions, 20 infants and 40 normal adults (Brain 1948).

The graspreflex (gr.r.). The adequat stimulus for the full reaction is dual. The first essential is a distally moving deep pressure over a specific area or the palmar surface of joints in the hand, which elicits a rapid brief finger flexion (the "catching" phase), which develops in to a strong "holding" phase only if traction is made upon the tendons of the

flexor or adductor muscles thus thrown into preliminary contraction. The response is then maintained only by traction. The chief difference between the holding phase and the myostatic resistence of spasticity is that the gr.r. can be set up at any length of the finger flexors and is always self-sustaining.

The instinctive grasp reaction (i.gr.re.) is usually associated with the gr.r. The adequate stimulus is a stationary contact within the palm, which elicite a closure of the whole hand made in a series of small movements. When stimulating the dorsum of the hand the closure of the fingers is preceded by a more elaborate series of movements (the "closing reaction") each of which brings further contacts with the stimulus and brings the stimulus closer to the palm. Movements of the stimulus will lead to movements of pursuit ("magnet reaction") or to groping in space. Movement of the stimulus once confined within the palm leads to an immediate tightening of the grip ("trap reaction"). Grasping in response to a visual stimulus is an extension of the tactile i.gr.re. The gr.r. and the i.gr.re. are both more easily provoked when the attention of the patient is distracted. The i.gr.re. has complete analogy with the instinctive sucking reaction and the instinctive visual fixation reaction, which together with "gegenhalten" by passive movements, Mayers reflex and the "bending reaction" are released by frontal cerebral lesions.

The bendingreaction examined by by the author is related to the Mayers reflex of the fingers and Leris forearm reflex and consist of a contraction of the elbow flexors during steadily bending of the wrist in radial direction, trying to increase the radial flexion out over the outermost position. The contraction is reinforced when trying to counteract the provoked elbow flexion by passive extention of the elbow joint. The same sort of reflex may also be elicited in the other joints in the upper extremities and partly also in the lower extremities.

The grasp reflex described above was found in the 20 infants examined. 10 of which were premature. A distinct gr.r. also could be elicited repeatedly in 5 out of 40 normal adults. Only in 14 of the adults no trace of grasping could be provoked during this examination. However, in most normal persons the grasping probably is developed when they are fully distracted, f.inst. in emergency. The study of patients and normals seems to show that the grasp reflex is a part of the voluntary movements. The bending reaction is very rare in normal persons.

SJÖSTRAND, TORGNY (Stockholm). **Determination of the total haemoglobin content of the body.**

A method for the determination of the total haemoglobin content of the body is described, involving the determination of the alveolar CO-concentration before and after the administration of 12—30 ml CO to the body. A special CO-meter is constructed for this purpose, which makes it possible to determine very low CO-concentrations to 0.0005 % in air or oxygen with an error of ± 2 %. The alveolar air specimen is obtained by rebreathing in a closed system filled with 90—95 % oxygen and fitted with a carbon dioxide filter and a rubber bag.

The accuracy of the Hb-determination on one and the same individual is comparable to a standard deviation of 3—4 % of the value and can be increased in several ways.

The total haemoglobin content of the body has been determined for 117 apparently healthy males, with an average total haemoglobin of 1.126 ± 0.0096 per cent of body weight and with a standard deviation of 9.2 %. Calculated on the body surface (according to DuBois' formula) the amount of haemoglobin in gram was 425 ± 3.6 per sqr.m. and a standard deviation of 9.2 %. On 69 women without any signs of blood or heart diseases the corresponding values were 0.86 ± 0.023 per cent of body weight and with a standard deviation of 11.9 %, resp. 321 ± 4.5 gram per sq.m. body surface and a standard deviation of 11.7 %.

The method has been used for determination of the haemoglobin content after a bleeding and the amount of haemoglobin formed per day.

The method is simple to employ and can be used for clinical purposes in cases of haemorrhage and shock for the determination of the degree of bleeding and for discovering of occult bleeding.

SKOU, J. CHR. (Aarhus). **Determination of procain in blood and spinal fluid.**

1. Extraction with ether and titration.

After adding alkali in surplus of 0.2—0.5 ccm of blood or spinal fluid you are extracting with ether for 4 hours. The extract is absorbed in 0.2 ccm 1/20 n HCl. The method of roching-extraction is used a. m. Widmark, in inversed V-formed glass tubes, Ørskov's model.

The extracted amount of procain is ascertained by titration with a microburette (Widmark-Ørskov) with 1/20 n NaOH.

The ascertained amount is procain + diethylaminoethanol from the contingently split procain, is consequently denoting the totally added amount of procain. The blind value of spinal fluid and blood is o.

Well fitted for aseertainment of series of coneentrations down to 1/1000 m.

2. Colorimetrical.

Aften diazotation and coupling with colourcomponent the intensity of colour is measured colorimetrically.

By direct ascertaiment on blood and spinal fluid is obtained procain + split procain ascertained as paraaminobenzoic acid.

After the extraction with ether, as shown above, you get in the extract the free amount of procain. By ascertainment of the rest is obtained the split procain ascertained as paraaminobenzoic acid.

Applicable at direct ascertainment of blood down to 0.2 mg %.

When extracting with ether it is however possible to concentrate, e. g. to extract 2 ccm of blood and absorb in 0.5 ccm of acid, or repeat the extraction with new blood, futhermore by diazotation on the extract you will avoid the attenuation that is necessary by direct ascertainment of the blood.

Well fitted for determination of series.

SKOUBY, ARNE P. (Copenhagen). **The aortic pressure in dogs during work.**

The aortic pressure variations are recorded from dogs running on a treadmill by means of a manometer with electrical transmission. In order to get a gauge for the metabolic rate the oxygen intakes have been determined at the different working rates using a mask and Douglas bag. The aortic pressure is determined during the transition from rest to work, the steady state at different working rates and the transition from work to rest.

The systolie blood-presure rises about 5 seconds after the beginning of work. In the course of 3—7 minutes it reaches a level, which with constant working-rate remains unchanged to the end of work and then after 0—3

seconds falls and reaches the resting level 3—14 seconds after the cessation of work. The increase in diastolic pressure is smaller, so that the pulse-pressure obtains higher values during work and decreases after cessation of work. The mean pressure remains constant or is insignificantly increased in the period after the beginning of work which precedes the rise in systolic and diastolic pressures.

The pulse-rate increases from the resting level about 0.8 seconds after the beginning of work, reaches a peak value in 10—25 seconds and settles with constant working rate at a constant level after about 3 minutes. At the end of work it falls suddenly with large deviations, but does not reach the resting level in the recording time (10—20 seconds). In the individual animal a linear correlation is found between steady state values of systolic and diastolic pressures and the corresponding oxygen intakes, while the connection between pulse-rate and oxygen intake is less well defined. The procentual increase in blood-pressure in 21 experiments on 5 dogs varies approximately linear with the oxygen intake per kg.

At the start of work the shape of the pressure-pulse curve changes, the systolic rise grows steeper and higher, the pressure fall from the maximum to the incisura increases and the diastolic fall is greatly diminished. These alterations appear before any change in blood-pressure level can be detected. The changes in the steep systolic rise depend on the pulse-rate.

The above results indicate among others, that the initial increase in pulse-rate is caused by other factors than impulses from the presso-receptors. The presso-receptors however partake in the regulation of the blood-pressure in the steady state, when the sensitivity of the circulatory centres has been changed probably by means of other simultaneous impulses.

STEN-KNUDSEN, OVE (Copenhagen). **Investigations on the mechanical anisotropy on the isolated frog muscle fibre.**

Simultaneous determination of the torsional rigidity and the longitudinal stiffness can provide information about the forces arising from the interaction of the minute structural elements in a striated muscle fiber during stretch and contraction.

Torsional elasticity is determined by measuring the couple induced in a torsion wire, when the isolated muscle fibre is periodically twisted. Longitudinal tension and stiffness are measured by means of a con-

denser myograph, the latter by determining oscillation frequency.

The directional couple D (the couple producing the torsion angle 1 rad) shows an exponential increase when the muscle is stretched. During contraction the directional couple (D_c) exceeds the resting value (D_r) 40 times for small extensions, the difference decreasing with higher extensions. When regarded at the same tension D_c is twice D_r for smaller extensions. An increase in temperature of 25° C. produces a decrease in D_c of 45 per cent, while D_r is only reduced 17 per cent.

The directional couple of a substance merely consisting of longitudinally arranged elements without mutual interaction has a maximal value which is 30 times smaller than that observed in the muscle fibre. When the relation existing between the torsional and longitudinal elasticity in an isotropic body is applied, calculation of D from the longitudinal stiffness results in values which exceed 10 times those found in direct torsion experiments. Also changes in angular forces in long range molecules result in small additional torsional forces which can be disregarded. The directional couple must, therefore, be ascribed to linkages which join together parts of the molecules belonging to neighbouring chain molecules.

Investigations of the quotient stiffness/directional couple (S/D) as function of stretch is an expression of the degree of the mechanical anisotropy of the muscle fibre. S/D as function of the extension increases almost linearly up to 30 % of stretch and increases steeply with higher extensions. Thus, above 30 % of stretch the forces acting in the longitudinal direction of the fibre arise principally from intermolecular attractions rather than from changes in the statistical configuration of long chain molecules. Referring to the same tension, during contraction the ratio S/D is higher than at rest.

The finding of a torsional rigidity proper helps us to realize the idea of a system consisting of long chain molecules arranged in almost straightened condition, since transverse bindings act as if the long chain molecule consisted of a number of shorter molecules and a short molecule is more likely to be found near maximal length than is a long one.

STRÖM, GUNNAR and UVNÄS, BÖRJE (Lund). **The central nervous representation of gastrointestinal motor and secretory activity.**

Insulin-induced hypoglycemia causes gastric secretion in the dog. The secretory effect of hypoglycemia is believed to be due to activation of "vagal centres" in the brain.

In a series of dogs the gastric secretory response to insulin was determined. After decortication the secretory response remained unaltered. Frontal sections of the brain caudal to the hypothalamus reduced but did not abolish the secretory response. The extent of the operative procedures was controlled by autopsy.

Certain autonomic regions of the frontal lobe and of the hypothalamus are reported to influence gastrointestinal motor and secretory activity.

In a series of cats the frontal lobe was systematically explored with the use of the Horsley-Clarke technique. Stimulation of different regions of the frontal lobe was found to cause inhibition or activation of gastrointestinal motor activity. The stimulated regions were localized by histological sectioning.

SUOMALAINEN, PAAVO (Helsinki). **Insulin and hibernation.**

Certain mammals sink during the winter into a special state of torpor, called hibernation. The most characteristic feature of the physiology of hibernation is the change in an animal from homoiothermy to poikilothermy. For instance, the body-temperature of the hedgehog can fall to + 1.5° C. during hibernation. Another special feature of hibernation is a strong hypoglycaemia whilst the glycogen store of the liver remains unchanged. When the animal wakes up, poikilothermy changes into homoiothermy again, the body-temperature rising in a cople of hours to about 35° C., glycogen breaking down into lactic acid and the precentage of blood-sugar returning to the normal value.

The islets of Langerhans are relatively primitive in structure in the hedgehog. They are very irregular in form: there is no sheath of connective tissue surrounding them, and therefore the border between the islets and the exocrine tissue of the pancreas is often very indefinite, especially as outgrowths from the islet tissue are pushed far into the exocrine tissue. Histological investigations show that the islet tissue of the pancreas is very hypertrophied during hibernation in the hedgehog. As the cells of the islet tissue are microscopically of the same sort during hibernation as during the summer,

there is good reason to assume that the secretion of insulin has increased during hibernation. As the secretion of adrenalin is at the same time at a minimum, we have a natural explanation for the hypoglycaemia typical of hibernation. The increased secretion of insulin during hibernation also explains the experiments in which the author brought about artificial hibernation. Then insulin was injected into the hedgehog in the summer and the animal was put into a refrigerator. It thereupon sank into a state of torpor, which in all essential respects resembled natural hibernation. Amongst other things, it changed into a poikilothermic animal.

SVAETICHIN, GUNNAR (Helsinki). **Electrophysiological Investigations of Single Nerve Cells.**

A micro-electrode technique with a simultaneous microscopic inspection has been used which has made it possible to use electrodes with a diameter down to one micra and simultaneously to localize the position of the electrode on the surface of a living cell. An electrical mapping out of the perikaryon of spinal ganglion cells of frog has been carried out, by recording action potentials from about thirty points on the surface. When only one cell is in action, variations occur in the shape of the impulse recorded from the perikaryon, which suggests that the impulse is composed of different components. Experiments have been performed in order to localize these components to different parts of the cell. The relation between the diameters of the axon and perikaryon, and the amplitude, form and other characteristics of the impulse has been investigated. There exists an obvious difference between the characteristics of cells belonging to different types of receptors.

SVEINSSON, SVEIN L. (Oslo). **Effects of Insulin on Adrenalectomised Rabbits.**

The line of reasoning which forms the basis for these experiments is that animals from which the adrenal glands have been removed are better suited for demonstration of the actual mode of action of insulin than animals with intact adrenals, since neither the adrenalin nor the hormones of the adrenal cortex will then be able to influence the results of the experiments.

The experiments, which were carried out in 1940,[1]) con-

[1]) Sveinsson, Svein Lunde. Avhandlinger utg. av Det Norske Vidensk.-Akad., Oslo, I. Mat.-Naturv. Klasse 1941, No. 12.

sisted in recording the consumption of oxygen and the production of carbon dioxide during administration of insulin and of insulin plus glucose to rabbits narcotised by pernocton. The animals were partially immersed in a water bath of constant temperature and connected with a closed-circuit respiration apparatus by means of a tracheal canula. The excretion of carbon dioxide was measured during several hours in periods of 30 to 60 minutes, while the oxygen intake was recorded in periods of 10 to 15 minutes.

Administration of insulin to animals with intact adrenals leads to an increased consumption of oxygen, a somewhat greater increase in the excretion of carbon dioxide and a consequently increased R.Q. Administration of insulin to animals which have been adrenalectomised immediately before the experiment leads, on the contrary, to reduction of the oxygen consumption, to a somewhat smaller reduction in carbon dioxide production, with resultant increase of the R.Q. In some experiments the consumption of oxygen declines all the time until the animals die from stoppage of respiration. No definite relation between the blood sugar level and the oxygen consumption has been observed.

In a number of experiments insulin plus glucose was given, with the result that the consumption of oxygen, after a temporary rise, falls to values still lower than those attained after insulin alone. Also during administration of insulin plus glucose the R.Q. increases and in several periods reaches values above 1.0.

The effect of insulin on adrenalectomised rabbits here observed does not harmonise with the usual conception of the action of insulin, namely, the supposition that insulin promotes the combustion of carbohydrates and augments the formation of glycogen. In these adrenalectomised animals the most striking effect of insulin is a reduction of the oxygen consumption, and the high R.Q. values observed seem to indicate that anabolic processes, such as formation of fat from carbohydrates, are stimulated.

TARVAST, M. O. (Helsinki). **The influence of age on the cornea-sensibility.**

TEORELL, TORSTEN (Uppsala). **Some view points on electrical tissue polarization.**

It is well known that the polarization of tissues can appear as variations in the impedance towards A.C. of differ-

ent frequencies or as changes in the tissue potential. In recent years impedance measurements with A.C. have been frequently employed for the characterising of the polarization behaviour. A new method employing square wave currents instead of sinusoidal currents makes it possible to record impedance changes of a very short duration, which appear in tissues, for instance after brief electrical stimuli. The results may be expressed either as a "time-current strength" relationship or in terms of an "equivalent scheme" based on resistances and a "polarization capacity" (impedance loci diagrams).

Excised surviving frog skin has been employed as a tissue, the inside being bathed in a nutritional solution, the outside subjected to various electrolyte solutions. The skin could also be electrically "stimulated" by aid of Ag-Pt electrodes placed across the skin. The skin potential was measured via calomel electrodes. The "square wave analysis" employed has been recently described.[1]) Some experiments are described: (1) Ringer, NaCl, KCl, and $CaCl_2$ have been compared as outside solutions. They increase the D.C. resistance (i.e. the "polarization resistance") and decrease the potential in the order given. The changes of the polarization capacity will be discussed. (2) Rectifying properties of the skin can be demonstrated. (3) The influence of the strength, duration, and current direction of D.C. stimuli has been studied. Most stimuli will cause a temporary decrease of the D.C. polarization resistance (the high frequency resistance remains rather constant). The "polarization capacity" seems, however, to remain unaltered. There are, in general, marked differences in the effects of "anodic" and "cathodic" stimulation, in particular as regards the skin potential.

The frog skin results have been compared with polarization phenomena and potentials exhibited by some artificial membranes as studied by the present technique. It seems probable that a great deal of what is here termed as "rectification" and "polarization" can be related to i o n i c d i s t r i b u t i o n p h e n o m e n a in charged membranes.

TUURALA, OSMO (Helsinki). **Einige Züge aus der Physiologie des Insektennetzauges.**

E x n e r (1891) teilt die Netzaugen der Insekten auf Grund der Bildungsweise des in ihnen enstehenden Bildes in Appositions- und Superpositionsaugen ein. Bei den ersteren

[1]) Acta Physiol. Scand. *12*, 235 (1946).

ist die optische Isolation der Ommatiden eine vallständige, und es haben also zu den Rhabdomen nur die in der Richtung der Ommatidienachse eintreffenden Lichtstrahlen Zugang. Beim Superpositionsauge empfängt ein jedes Rhabdom Lichtstrahlen durch mehrere Kristallkegel, wenn das Auge auf Dämmerung eingestellt ist und das Pigment zwischen den Kristallkegeln lagert, so dass die Ommatiden ohne optische Isolation bleiben. Bei Lichteinstellung des Auges wandert das Pigment proximalwärts und bewirkt eine Isolation der Ommatiden. Das Auge betätigt sich dann wie ein Appostionsauge.

Vortragender hat die Bewegungen des Pigments im Netzauge der Schmetterlinge untersucht. Bei den Schmetterlingen wandert Irispigment und das Pigment der Retinulazellen bei Licht proximalwärts und bei Dunkelheit distalwärts. Ausserdem vollziehen sich Pigmentbewegungen oft auch in den Basalzellen, doch sind die Bewegungen hier denjenigen der vorhergehenden entgegengesetzt. Ferner wandern im Auge der Schmetterlinge allgemein auch die Retinulazellkerne, und zwar in Übereinstimmung mit dem Irispigment.

Die Wanderungen des Pigments in Licht- und Dunkelstellung können bei den Schmetterlingen auch in einem exstirpierten Auge zustandegebracht werden. Dies bezeugt, dass sich die fraglichen Bewegungen ohne zentralnervös-hormonalen Einfluss abspielen können. Demnach haben wir als Primärvorgang offenbar eine photochemische Reaktion, als dessen unmittelbare Folge eine die Pigmentwanderungen bewirkende Reaktionsserie ausgelöst wird, die von der Temperatur abhängig ist. Die Pigmentwanderungen werden jedoch zum Teil auch durch die zentral-nervös-hormonale Regulation beeinflusst, die es bewirkt, dass das Pigment in den Augen permanent im Dunkeln gehaltener Schmetterlinge tagsüber in Lichtstellung und zur Nacht in Dunkelstellung wandert. Der Rhytmus dieser Bewegungen ist dabei offenbar wenigstens zum Teil von der Aktivität der Tiere abhängig, da sich im Dunkeln tag-eingestellten Augen durch Versetzen der Tiere in lebhafte Bewegung in Dunkeleinstellung bringen lassen.

WALAAS, O. and WALAAS, E. (Oslo). **Phosphorous compounds in uterine muscle.**

The content of the energy-rich phosphate compounds, adenosin-triphosphate and creatin-phosphate in uterine muscle has been investigated in rats and in rabbits. Further the amount of anorganic phosphate, total acid-soluble phosphate and glycogen has been studied.

The content of the two energy-rich phosphate compounds in uterine muscle is very low. Adenosin-triphosphate is present in a concentration of 2—3 mg per cent in rabbits and about 5 mg per cent in rats. The concentration of creatin-phosphate is only about 1 mg per cent. These values are far below those found in cross striated muscle tissue. The amount in uterine muscle in relation to cross striated muscle is calculated: creatin-phosphate 1:50, adenosin-triphosphate 1:25 in rabbits and 1:10 in rats.

The content of anorganic phosphate is about the same in the two types of muscle tissue.

The significance of the substances studied in contraction of smooth muscle will be discussed.

Further the hormonal effect on the phosphorous compounds of uterine muscle has been studied. In rats the following grops have been investigated: 1) Ovariectomized animals, 2) Ovariectomized rats injected with estradiolbenzoat. Immature rabbits have been studied partly after injection of estradiolbenzoat, and partly after estradiolbenzoat + progesterone.

No definite effect on the content of anorganic phosphate, creatin-phosphate, adenosin-triphosphate, total acid-soluble phosphate has been found by the various hormonal stimuli. On the contrary the glycogen content of uterine muscle shows an increase after injection of estradiolbenzoat. This hormone produces an increase in the glycogen concentration 3—6 times the value found in castrated animals, and lies above the glycogen content of cross striated muscle. The increased energy transformations in uterus after estradiol treatment therefore to a great degree probably must depend on glycogen breakdown.

It is assumed that the low content of the energy-rich phosphate compounds in uterine muscle has some relation to the contractility of smooth muscle, with the slowness in contraction and the still slower relaxation.

von WENDT, GEORG (Helsinki). **Vitamin-studien.**

Der Vortrag beleuchtet das Abhängigkeitsverhältnis zwischen A- und C-Vitamin und ganz besonders die Unfähigkeit des tiersichen Organismus ohne hinreichende Zufuhr von Vitamin A genügend oder überhaupt Vitamin C aufzubauen. Hierbei wird auch die C-Vitaminsynthesen im menschlichen Organismus besprochen wie auch unser damit zusammenhängdes Bedürfniss an Vitamin A.

Die Untersuchungen des Redners wie auch die von Pro-

fessor Giroud über die Bedeutung des Vitamin C:s für die Muskelarbeit werden durch Lichtbildern anschaulich gemacht, so auch die Untersuchungen des zuletzt genannten Forschers über den Einfluss der Muskelarbeit auf den C-Vitamingehalt der Nebennierenrinde wie auch die Erklärung der Veränderungen.

Der C-Vitamingehalt der Nebennierenrinde verschiedener Tierarten, wie auch der von Menschen und menschlichen Foeten wird mit Hilfe von Lichtbildern verglichen und hieraus zu ziehende Schlüsse erwähnt und diskutiert.

Zuletzt wird die Frage ob die Versorgung ganz besonders mit den oben erwähnten Vitaminen Beziehungen zur Widerstandskraft des Organismus hat und damit zusammenhängende Fragen gestreift.

WEIDMANN, SILVIO (*Uppsala*). ***The recovery process in Nitella after make stimulation and after break stimulation.***

Nitella, a giant water alga, possesses bioelectrical properties most similar to those found in nerve and muscle (excitability, conduction of impulses). One single cell of Nitella reaches a diameter of half a mm and a length of several cm. These dimensions together with desirable electrical data make Nitella a most suitable object for bioelectrical studies.

A local exitation in Nitella may be started either by the make of a cathodal current (cathode placed on the outer surface of the cell's membrane, anode at a KCl depolarized end), or by the break of an anodal current. Depending on whether make stimuli or break stimuli are used, remarkable differences in the recovery process can be observed.

Membrane potential and membrane impedance are registered simultaneously. The records show that when using anodal break stimuli instead of cathodal make stimuli

a) the recovery process goes on at a lower speed,
b) the action potential reaches a higher maximum value,
c) the membrane impedance drops to a lower minimum value,
d) all these features are further stressed by stimulating with anodal currents of a longer duration or of a higher strength.

Whilst cathodal currents sent through the membrane during recovery do not detectably slow down the process of

restitution, it is possible to keep the membrane almost on the hight of excitation (as judged from potential and impedance values) by applying pulses of anodal current in suitable intervals.

It seems impossible at present to interpret the observations in more definite terms than perhaps as follows: The displacement of charged particles across the membrane under the influence of an anode creates conditions, which — after the break of the polarizing current — tend to stabilize the state of excitation and render recovery more difficult.

WRETLIND, K. A. J., Stockholm. **The effect of amino acids on growth.**

The effect of an amino acid preparation on the growth of premature infants.

It is well known fact that it is difficult to get a good gain of weight during the first month of life in premature infants. However it has been shown that if one gives amino acids as a supplementary food to the premature infants one immediately gets a good gain in weight. (Magnusson 1944, Wretlind 1945, Jorpes, Magnusson & Wretlind 1946.)

In these investigations an amino acid preparation called aminosol has been used. It is prepared from casein, which first is hydrolyzed with trypsin and erepsin and afterwards the product is dialyzed in order to free it from non-digested proteins and highmolecular peptides. The preparation, thus obtained contains about 76 % free amino acids. The nitrogen content is 12 %. It has no anaphylactic power. The lethal dose (LD 50) of the preparation has proved to be 12.5—15 g per kg body weight. (Goldberg and Wretlind 1947.)

Per kg and day 2.5 g of Aminosol have been given as a supplementary food to the premature infants, which have received breast milk to supply the body with about 100 kcal per kg and day. The feeding of this mixture of amino acids produced in every instance a distinctly higher gain in weight than breast-milk alone. To eliminate as far as possible the high variability which has always to be taken into account at this stage of life, it was arranged that the same infant was given alternate feeds of breast milk alone and of breast milk to which the amino acid mixture had been added. In all the tests, where the effect of the amino acid mixture was com-

pared with that of undigested casein there was a much larger gain in weight, while the infant was receiving the amino acid mixture. In other words the premature infants cannot assimilate, at any rate completely, the undigested casein, because the digestions glands are not probably fully developed.

The addition of amino acids to the breast milk is of most value during the first weeks of life. Amino acids have been given to premature infants of different birth weight during the first month of life. The gain in weight during this time has in general been about 2 times as great as the gain in premature infants of the same weight groups, which received only breast milk.

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The effect of essential, synthetic amino acids on the growth of rats.

By Rose (1938) it has been shown that only ten amino acids are essential for the growth of rats. With a diet containing these ten amino acids as the sole source of nitrogen it is possible to maintain growth. In these previous experiments the amino acids used were always to a part prepared from protein. Thus the natural isomer and racemic form were used alternately. It has been shown that certain isomers of the amino acids cannot be utilized by the body. The question then arises whether the isomers that cannot be utilized by the body are toxic or harmles to the organism. Investigations on this subject have not previously been made. Should it be so that the organism can develop on a diet containing a mixture of the 10 essential amino acids solely supplied in the racemic forms, this would signify that growth can be obtained with purely synthetic amino acids. In the investigation reported here it has been shown that with the 10 essential amino acids, synthetically produced, as the sole source of nitrogen, growth in rats can be promoted.

The amino acids used were all synthetized by the author (Wretlind 1948). From these ten amino acids, dl-argininc, dl-histidine, dl-isoleucine, dl-leucine, dl-methioninc, dl-phenylalanine, dl-threonine, dl-tryptophane, dl-lysine and dl-valine a mixture was made in about the same proportion as

the amino acids occur in casein. The nitrogen-free diet contained merely 0.002 % N. The rats daily got an addition of the watersoluble vitamins. As experimental animals, white rats weighing 44.5—50.4 grams were used. The experimental period was in general 10 days.

On a diet containing 10 % of the said amino acid mixture a growth averaging 0.33 grams per day was obtained.

On a 20 % diet the growth of the rats averaged 0.85 grams per day.

On the other hand if the rats received 30 and 40 % amino acids in the diet, a decrease in weight, averaging 0.1 and 0.7 grams a day respectively resulted.

For the first time it has thus been shown that it is possible to maintain growth with wholly synthetically made amino acids. In higher concentration these amino acids, however seem to have some toxic power. Which of the amino acids or isomers produce this effect, cannot be ascertained without thorough investigation.

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ZOTTERMANN, YNGVE (Stockholm). **Afferent impulses from the heart.**

ÖBRINK, K. J. (Uppsala). **Carbonic anhydrase and the permeability properties of the gastric mucosa.**

The gastric mucosa has the power of accumulating the chloride ions of the gastric contents to a level much higher than the concentration in the blood plasma. This accumulation is greater than could be expected from the Donnan effect and occurs without the aid of any secretory process, i.e. in a resting stomach.

A theory was recently advanced [1]) which attempted to explain this phenomenon: Bicarbonate ions diffusing in the direction blood-stomach were throught to form carbonic acid by de-ionisation in the mucosa. The carbonic anhydrase in the mucosa cells was thought to convert this acid into carbon dioxide and water. In order to preserve electric neutrality another anion (Cl^-) has to leave the mucosa. Thus the anion

[1]) Öbrink, K. J., Acta Physiol. Scand. 1948, *15*, Suppl. 51.

which entered the mucosa (HCO_3^-) has probably been substituted by the Cl^- ion. This result is quite analogous to the chloride shift in the red blood corpuscles.

The chlorides entering the stomach may therefore originate from two separate sources, a) the Cl^- from the plasma and b) the Cl^- from the mucosa which are substituted for the HCO_3^- from the plasma. The diffusibility of HCO_3^- through the mucosa is supposed to be much (or entirely) depressed by the action of carbonic anhydrase.

This explanation for the chloride accumulation has been supported by some experiments where the effect of carbonic anhydrase was abolished by thiocyanate and by others where the bicarbonate concentration in the plasma was changed.

ØRSKOV, SØREN L. (Aarhus). **Experiment on Potassium Absorption by Yeast.**

First it was tried if yeast cells will absorb potassium from the surrounding fluid, when a hypertonic NaCl-solution is added, as is the case with Coli bacilli.[1])

In some experiments a minor rise of the potassium concentration in the cells occurred, but though many experiments were performed it was not possible to make out the reason of the differing results.

At the same time some experiments were performed where different organic substances were added to a yeast suspension and the potassium concentration of the outside fluid was followed.

Verzar and Pulver (1940)[2]) showed that glucose when added to washed and aerated yeast causes a considerable K-absorption.
has the same ability. If KCl is the only salt in the outside solution the reaction will become more acid, indicating that Cl is absorbed to a much less degree.

35 substances were added and besides glucose and ethyl alcohol, it was found that propyl alcohol, butyl alcohol, acetaldehyde and acetic acid give the same change of pH and K-absorption.

The concentration of the substances in the solution is

[1]) ØRSKOV, S. L.: Acta path. et microbiol. Scand. 1948.

[2]) VERZAR, F. and PULVER, R.: Nature. 1940. *146*. 823.

[3]) ROTHSTEIN, A and ENNS, L. H.: J. cell. and comp. Physiol. 1946. *28*. 231.

about 40 mg %, but 4 mg % of the above-mentioned substances is enough to produce a shifting of pH. The volume of cells is about 12 % of the solution.

Glucose in most experiments produces a less pronounced shifting of pH than the other active substances and after about 10 minutes it declines and potassium will pass out of the cells.

If in such experiment the suspension is aerated with air containing 20 % CO_2 the outer fluid will become more acid and stay so for much longer time. Probably the explanation is to be put in relation to the pH of the cell.

Demonstrations:

1. BIERRING, E. & E. NIELSEN (Copenhagen): A Danish flame-fotometer for sodium and potassium analyses.

2. SEYFFARTH, H. (Oslo): The grasping phenomena in patients with cerebral lesions and in normal persons.

3. TEORELL, T. (Stockholm): Some view points on electrical tissue polarization.

4. ÅKERBLAD, B. (Stockholm): A Swedish built Millikan-oximeter. Discussion of results.

5. ÅKERBLAD, B. (Stockholm): The use of thermoresistors in the measurement of temperature and its use in physiology.

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STUDIES ON THE CHEMICAL DETERMINATION, OCCURRENCE AND METABOLISM OF ADRENALINE IN THE ANIMAL ORGANISM

BY
AIMO PEKKARINEN

HELSINKI 1949

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PREFACE.

It gives me great pleasure to express my deepest gratitude to the Head of the Department of Medical Chemistry of Helsinki University, Professor P. E. SIMOLA, to whom I am indebted for my subject, for his invaluable help and support during my study which has been greatly promoted by his advice and guidance at various stages of my work.

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Helsinki, in May 1948.

Aimo Pekkarinen.

INTRODUCTION.

Simultaneously with the development of hormone chemistry attempts were made, in order to gain knowledge of the physiological significance of hormones, to determine them by quantitative methods as soon as the physiological and chemical characteristics of the hormones became known. Endeavours have been made to build up methods by means of which small effective quantities of the substance can be demonstrated in biological material. This also applies to vitamins. Originally the methods were in general biological, since these substances are more easily demonstrable by means of their biological effects than by chemical means. Biological methods are usually more specific than chemical ones, but difficult and complicated, and their accurate measurement is often comparatively inexact. Therefore biological methods have been abandoned, and endeavours have recently been directed towards a better development of chemical methods for the determination of hormones and vitamins.

To what extent a chemical determination of hormones and vitamins is possible, is highly dependent on the chemical nature of the compounds. The chemical structure of several vitamins has such peculiarities, that many of them can be comparatively exactly determined by chemical means. It is quite clear that those hormones which are proteins with large molecules offer but small possibilities of chemical determination. The state of things is different where hormones with small molecules are concerned, such as hormones of the steroid type secreted by the suprarenal and sexual glands. Here the theoretical preliminary conditions

for a determination of these hormones exist.. Mention can be made as example of the positive results obtained in the quantitative determination of the 17-ketosteroids in urine.

Adrenaline, as a reactive substance consisting of small molecules, belongs to those substances which, in theory, can be determined in the organism by chemical methods, and therefore endeavours have been made for scores of years to develop different methods. But they have all been hampered by the weak specificity of the reactions.

Among the numerous reactions that have been made use of for the chemical determination of adrenaline, the fluorescence reaction appearing in an alkaline solution doubtless possesses fairly high specificity. Up to the present, however, this reaction has been comparatively little investigated with regard to the practical application. Since this reaction seems to offer better opportunities, owing to its sensitive and specific character, for a chemical determination of adrenaline than many earlier methods, and since a quantitative adrenaline determination still seems to be a matter of interest, it is the aim of this work to subject the quantitative adrenaline determination by the fluorescence method to a detailed investigation.

The purpose is to demonstrate what possibilities there are in general for a chemical determination of adrenaline in the blood, the results obtained by means of chemical reactions being highly contradictory. By means of the fluorescence reaction appearing in an adrenaline solution after addition of alkali, endeavours have been made to develop as sensitive a technique as lies within the limits of possibility. With the aid of a method based on the adsorption of adrenaline, an attempt has been made to determine the adrenaline content in peripheral and central blood. In addition researches have been made on the physiological properties of adrenaline in animal organism, by directing attention towards the appearance and disappearance of the injected adrenaline, and towards those factors which have some influence in this respect. It has also been the author's purpose to expose the possibilities of determining adrenaline not only in the blood but also in the tissues, and to study the factors affecting the loss of adrenaline by means of *in vitro* tests.

I.

DETERMINATION OF THE ADRENALINE CONTENT OF THE BLOOD.

Earlier Investigations.

Suitable methods of adrenaline determination have been continually sought for a period of about 50 years. It was found already a hundred years ago that the medullary portion of suprarenal glands stained with iodine and chromates. When, towards the close of the last century, a better knowledge was gained of the biological characteristics of adrenaline and at the beginning of this century its structure was made clear and it could also be produced synthetically, a lively interest was aroused in this hormone. Endeavours were made to determine adrenaline both by biological and by chemical methods. By and by knowledge was also obtained of its chemical reactions.

It is deemed advisable to begin by making a survey of investigations carried out by biological methods, since they were used at first, and then to report researches made by chemical methods, these latter having come into use at a later date. Table 1, appended to the end of the historical survey, page 20, for clarification, presents a summary of the more important results obtained earlier by biological and chemical methods.

1. Biological methods.

A. *Effects of adrenaline on the whole organism.*

Rise in blood pressure.

BATTELLI (1902 b) was the first to attempt a determination of adrenaline in the peripheral blood by physiological methods. By studying the rise in blood pressure produced by an intravenous serum injection, he

obtained an adrenaline content of 5—10 μg % in dog blood. The same method has also been used by WEISS and HARRIS (1904). It was proved deficient by later investigations (FRAENKEL, 1909). GLEY and QUINQUAUD (1913) could not determine by this means any adrenaline in the peripheral blood, but they found it in the blood of the suprarenal vein. Nowadays decerebrated test-animals are used. According to DREVON and VANSTEENBERGUE (1946) the blood pressure is not exactly proportional to the amount of injected adrenaline.

B. *Effects of adrenaline on different organs.*

Attemps were soon made to study in physiological tests the effect of adrenaline not only on the whole organism, but also on different organs: pupillary dilatation in an enucleated frog eye (EHRMAN, 1905, MELTZER, 1909), contraction of the rabbit uterus (FRAENKEL, 1909), constrictions of blood vessels in the lower extremities of the frog (LAEWEN, 1904, TRENDELENBURG, 1910), constriction of the cow arteries (MEYER, 1906), constriction of the rabbit auricular artery (KRAWKOW, 1913, PISSEMSKI, 1915) and immobilisation of the rabbit gut (CANNON and de la PAZ, 1911). The method with the rabbit's intestine was the one most in use. It also proved to be fairly specific.

a) Pupillary dilatation in an enucleated frog eye.

Several workers were unable to demonstrate by the frog eye method any adrenaline in venous or arterial blood (EHRMANN, 1905, SCHLAYER, 1907, WIESEL and SCHUR, 1907, FALTA and IVCOVIC, 1909, BORBERG, 1912, KAHN, 1909, 1912 a, b, BRANDT and KATZ, 1933 a, b, KATZ, 1933, BLOCK, 1933) but it was found in the blood of the suprarenal vein (EHRMANN, 1905, BORBERG, 1912), of the inferior vena cava (WATERMANN and SMITH, 1908), in the blood of a patient in hypoglycemia, shock (BRANDT and KATZ, 1933 a, b) or narcosis (WIESEL and SCHUR, 1908). Positive values were found in peripheral blood in nephritis (WIESEL and SCHUR, 1907, KAUFMANN and MANNEBERG, 1907, GOLDZIEHER and MOLNAR, 1908, EICHLER, 1907), but EHRMANN (1908, 1909), WATERMANN and BODDAERT (1908) considered them unspecific due to the pyrocatechin and other similar dilating substances. According to BORBERG (1912) the pupillary method is unreliable, capricious and insensitive (sensitivity 30 μg %), but the serum substances do not interfere with this as with many other methods. ROGOFF (1937) has developed a sensitive method for estimation of adrenaline by pupillary dilatation of the cat's eye, with a sensitivity of 0.5 μg %. According to SCHNEIDER (1922) adrenaline can be obtained with the pupillary method only in the suprarenal vein and not in arteries.

b) *Uterine contraction.*

The rabbit uterus method applied by FRAENKEL (1909), disclosed 250 μg % adrenaline in the peripheral blood of rabbits and cats, but O'CONNOR (1912 c) was unable to confirm this result and only found adrenaline in the suprarenal vein. ADLER (1914) found 8—14 μg % adrenaline in the suprarenal vein, but not in normal peripheral blood, only in thyrotoxicosis 5—8 μg %. FALTA and FLEMING (1911) considered substances found in the blood with the uterus method to be unspecific.

c) *Constriction of vessels.*

1. Constriction of frog leg vessels. O'CONNOR (1912 a, b, c), KAHN (1912 a, b), TRENDELENBURG (1910, 1916, 1923 a) and FLEISCHHAUER (1913), HESS (1922), HÜLSE (1922 a, b, c) and VOLHARD (1923) were unable to find any adrenaline in peripheral venous blood, but TRENDELENBURG found it in arterial blood 0.1—0.5 μg % and in the blood of the inferior vena cava, if the secretion of adrenaline was strongly increased, even up to 2.5 μg %, as well as in the blood of the suprarenal vein up to 20 μg %. He maintained that the true adrenaline content was even less than what he had found by means of his method, and he considered the constriction of vessels as partly due also to substances other than adrenaline. The same result was also arrived at by O'CONNOR (1912 a, b, c). The sensitivity of this method is according to TRENDELENBURG (1916) 0.1 μg %. In stored citrated blood there appeared substances with a contracting effect on vessels, which interfered with the determination of adrenaline (TRENDELENBURG and BRÖKING, 1911). According to FREUND (1920 a, b) they also form when thrombocytes disintegrate. STUBER, RUSSMAN and PROEBSTING (1923) considered the method of Trendelenburg unfit for the quantitative determination of adrenaline. Vasoconstricting substances were found in normal cases, hypertonia and malignant renal arteriosclerosis (KURÉ, NAKAYA, MURAKAMI and OKINAKA, 1932, 1933), in fever (v. EULER, 1926) and in hypoglycemia (HEILBRUNN and LIEBERT, 1939).

2. Constriction of cow arteries. MEYER (1906) found in cat serum and SCHLAYER (1908) in human serum substances similar to adrenaline having a constricting effect on the vessels, but ROTHLIN (1920 b) was unable to support these findings. He showed that many factors, such as vessel tonus, temperature and oxygen tension had a disturbing effect on the arterial method. The comparatively low sensitivity of the method (1—10 μg %) makes it unsuitable for quantitative work.

3. Constriction of rabbit ear vessels. SCHLOSSMANN (1927), KAHLSON and v. WERZ (1930), HARTWICH and HESSEL (1931 a, b) as well as BRANDT and KATZ (1933 a, b) and KATZ (1933) were unable to find any adrenaline in peripheral human blood by means of the rabbit ear-

vessel method, in spite of its sensitivity (0.0001 μg %), but SCHLOSSMANN (1927) was able to find at operation, after sugar- and strychnine injections all but 0.01 μg % adrenaline, in deep asphyxia 0.1—2 μg % and after a nicotine injection 2 μg %, SCHLOSSMANN and MÜGGE (1929) during anæsthesia 0.04 μg%. KAHLSON and v. WERZ (1939) found some adrenaline in patients suffering from hypertonia, thyrotoxicosis and fever, as well as BRANDT and KATZ (1933 a, b), MEYTHALER and WOSSIDLO (1935) in hypoglycemia and shock. The use of a normal saline solution containing serum as perfusion fluid renders the rabbit ear vessel method sensitive, but fluctuations of temperature interfere with the determinations, since adrenaline has a dilating effect already at 41°C. According to HARTWICH and HESSEL (1931 b) there are in the blood only unspecific constricting substances, which are found in the same amount also after extirpation of the adrenals. Adrenaline can easily be determined after an injection in the arteries but not in the veins in such amounts, which affect the blood pressure (HARTWICH and HESSEL, 1931 b).

d) *Immobilisation of the gut.*

By the rabbit gut method CANNON and HOSKINS (1911) and BRANDT and KATZ (1933 a, b) could not find any adrenaline in normal circumstances but adrenaline-like substances could be demonstrated after certain secretion stimulants. STEWART and ROGOFF (1917) found 0.4—1 μg % of these substances and BARRE and HOUSSA (1932) as well as BRANDT and KATZ (1933 a, b) some adrenaline in hypoglycemia. OKAMURA (1938) found 0.5—2 μg % adrenaline.

In the course of the first three decades of the 20th century physiological methods were used almost exclusively for the determination of adrenaline in peripheral blood. Of late, however, these methods have fallen out of use, researches having proved that its content is exceedingly small in peripheral blood, and that the earlier methods by means of which high adrenaline values were obtained are nonspecific.

Of late physiological methods have been used exclusively for determining adrenaline in the suprarenal vein, when studying the secretion of adrenaline from the suprarenal gland. The earlier results of these studies are presented in connection with the passage dealing with adrenaline secretion on page 72 (table 2).

2. Chemical methods.

A. Colour methods.

Nearly one hundred years ago VULPIAN (1856) found in the suprarenal medulla a substance, which gave a green colour with ferric chloride and a red colour with iodine and mercuric chloride. HENLE (1865) showed

somewhat later that one obtained there a dark-brown colour with potassium bichromate. After this, attempts were made to determine adrenaline quantitatively on the basis of these histological reactions.

a) *Oxidation.*

Many of the chemical reactions of adrenaline depend on its ready oxidation to a red-coloured compound. ABELOUS, SOULIE and TOUJAN (1905) used as oxidisers iodine, FRÄNKEL and ALLERS (1909) potassium iodate (sensitivity 330 μg %), COMESSATTI (1908) mercuric chloride (250 μg %), ZANFROGNINI (1909) potassium permanganate (100 μg %) and EWINS (1910) potassium persulphate (20 μg %). The sensitivity of the potassium persulphate reaction was found in later tests to be only 1000 μg % (BARKER, EASTLAND and EVERS, 1932). The red oxidation product of adrenaline is formed also in the presence of copper sulphate, platinum chloride, potassium chlorate, hydrogen peroxide (FRÄNKEL and ALLERS, 1909), sodium nitrite (BORBERG, 1912), gold chloride (GAUTIER, 1912) and organic oxidisers (oxidases from potatoes, beetle blood, mushrooms) (BHAGVAT, 1938). All these reactions were used for the quantitative estimation of adrenaline in the suprarenal gland. However, they are not sufficiently sensitive or specific for the quantitative determination of adrenaline in the blood.

The iodine reaction was further developed by v. EULER (1933 b) and WELLER (1933). According to EHRLEN (1948 a, b) iodine oxidises adrenaline partly to adrenochrome and 2-iodine-adrenochrome, in which there is a considerable difference in the light adsorption. EHRLEN (1948 a, b) recommends therefore the use of potassium ferrocyanide as oxidising agent at a *pH* of 6, when adrenochrome alone is formed.

CHIKANO (1929) used the iodate method for the estimation of adrenaline-like substances in the blood. He maintained that the reaction was due, in addition to adrenaline, also to other similar substances, uric acid and other products of protein metabolism. JACKEROTT (1941), DREVON and VANSTEENBERGUE (1946) recommended the same reaction for the determination of adrenaline in pure adrenaline solutions.

In the course of these last decades the investigators of the chemical methods of adrenaline determination have directed their special attention towards rendering the reaction more sensitive and more specific. BAYER (1909) noted that the addition of sulphanilic acid intensified the sensitivity of the potassium iodate reaction up to 20 μg %, but at the same time the specificity of the reaction weakened since, e.g. pyrocatechin gave the same reaction. The sensitivity was further increased by STUBER, RUSSMANN and PROEBSTING (1923) by addition of mercuric chloride up to 2 μg %. They found relatively high amounts of adrenaline in the blood. The colour in this reaction depends also upon the temperature, pH, presence of oxygen and light, and is also caused by oxidation products of

adrenaline. VIALE (1933 a, b, 1934 a, b) has also used the potassium bi-iodate-sulphanilic acid reaction. According to VIALE (1930) precursors of adrenaline also give this reaction. To increase the specificity of the test, VIALE (1933 a, b, 1934 a, b) added formaldehyde to a control sample to distinguish the adrenaline from other unspecific substances giving the same reaction. VIALE and CROCETTA (1933) demonstrated by this method 70—250 μg %, DOGLIOTTI and CROCETTA (1933) 250—330 μg % adrenaline in the blood, CROCETTA (1933) 600 μg % adrenaline in the blood corpuscles. MACCHIARULO (1935 a, b) found in the blood of fetus 130—200 μg % and in the amniotic fluid 50—100 μg % adrenaline. According to DICKER (1934) Viale's reaction is, however, nonspecific, the reacting substances already producing an identical colour. According to BACQ (1932) and LOEBL (1936) the same reaction is also produced by the pyrocatechin derivatives. KONSCHEGG and MONAUNI (1936) showed 50 μg % adrenaline in the serum of man with the same reaction. VELICOGNA (1934) also used the sulphanilic acid iodate reaction.

b) *Reduction.*

BATTELLI (1902 a) seems to be the first to have attempted to determine the adrenaline content of the blood by means of a chemical method. He used a ferric chloride reaction, which, however, is much too insensitive (3000 μg %) and also unspecific. E.g. bilirubin and many other substances give the same colour as adrenaline. The pH also influences the colour formation. WIESEL and SCHUR (1907) showed positive results by ferric chloride reaction in nephritis.

Adrenaline reduces ammoniacal silver, potassium bichromate, manganese dioxide, phosphotungstic, arsenotungstic and arsenomolydbic acid. A new phase of development in the chemical methods of adrenaline determination was brought about by the methods last mentioned, based on the pyrocatechin group reactions. FOLIN, CANNON and DENIS (1912) used a phosphotungstic acid method for adrenaline determinations, which is also employed in uric acid determinations. Its sensitivity was 33 μg %. The same reaction was also used by AUTENRIETH and QUANTMEYER (1921). In it the colour diminishes somewhat rapidly. KOBAYASHI (1935) obtained by means of the phosphotungstic acid method 7.3 μg % adrenaline from rabbit-ear blood, and 32 μg % from carotid blood. JOHANNESSOHN (1916) considered oxidising as well as reducing chemical methods suitable only for determinations of adrenaline in pure adrenaline solutions, since several other substances can produce the same reactions. Nonspecific factors influencing the reactions are glutathione, uric acid, cysteine and ascorbic acid (FUJIWARA and KATAOKA, 1933, v. EULER, BURSTRÖM and HÄLLSTRÖM, 1932, GUHA, 1935, DEVINE, 1937).

In the course of the last decade adsorption methods have been developed for the elimination of nonspecific chromogenic substances, based on adrenaline adsorption to aluminium hydroxide or silicic acid at a slightly alkaline reaction. Several nonspecific factors can then be eliminated by first adsorbing them at a more acid reaction and thereafter by adsorbing adrenaline from a slightly alkaline solution.

Silicic acid adsorption.

The first to use the adsorption method in chemical determinations of adrenaline was WHITEHORN (1923, 1935), who adsorbed adrenaline on to silicic acid. He separated adrenaline with the help of silicic acid adsorption from non-basic and strongly basic reducing substances. After elution from silicic acid he determined adrenaline with the arsenomolybdic acid reaction. He succeeded in determining 2.5—7.5 μg % adrenaline in femoral arteria, and in inferior vena cava 17.5—25 μg %, if the secretion of adrenaline was heightened by stimulating the splanchnic or sciatic nerve. Yet he was unable to demonstrate any adrenaline in peripheral venous blood. DOPY and WEISINGER (1938) found by WHITEHORN's method 5—7 μg % adrenaline in the blood of the guinea pig. EICHLER and NOACK (1939) have criticised the adsorption of WHITEHORN's method as being deficient but KOBRO (1936, 1946 a), on the other hand, claims to have obtained quantitative results by means of the same method. KOBRO (1936, 1946 a) has developed further the filtering and colorimetry of the WHITEHORN method. According to KOBRO (1946 b) the normal adrenaline content in man is 2.8—7.9 μg % (the average being 4.4 μg %). In pathological states there are no great variations in content of adrenaline (KOBRO, 1946 c, d). False results in this method can be due to unknown substances which are formed during blood coagulation. These substances combine with silicic acid and yield the same colour as adrenaline (KOBRO, 1946 a). It is possible that in the analyses not only adrenaline, but also its preliminary stages and products of disintegration are determined (KOBRO, 1946 e). GIORDANO and ZEGLIO (1938, 1939) carried out, instead of protein precipitation, an adrenaline dialysis and adsorbed adrenaline on to silicic acid. By using the arsenomolybdic phosphotungstic acid reaction they obtained 40—317 μg % adrenaline content in the blood.

Aluminium hydroxide adsorption.

SHAW (1938) adsorbed adrenaline on to aluminium hydroxide at pH 8, after having eliminated under acid conditions glutathione and other reducing substances and determined adrenaline after elution with arsenomolybdic acid. He maintained that his method was more simple and sensitive

than that of WHITEHORN (1935), and that he could demonstrate by it 5 μg % adrenaline in rabbit and 1.6—2 μg % in human blood. SARFY (1938, 1939) determined by SHAW's method 6 μg % adrenaline in pigeon and guinea pig blood. TIETZ, DORNHEGGEN and GOLDMAN (1940) found by SHAW's method 100—160 μg % adrenaline in insulin shock 3—4 hours after injection.

BLOOR and BULLEN (1939, 1941 a, b) considered the adsorption by SHAW's method inadequate. According to BLOOR and BULLEN (1941 b) there are in the venous blood of dog and man 20—50 μg % adrenaline-like substances yielding colour with arsenomolybdic acid, but these substances are, in contradistinction to adrenaline, alkali-resistant. They considered that there is thus no adrenaline at all in venous blood. RAAB (1941 a, b, 1943 b, c) also regarded SHAW's (1938) method as nonspecific and considered that the colour with arsenomolybdic acid corresponding to 6.6—22.2 μg % (average value 15.6 μg %) adrenaline in the blood is due to ascorbic acid and adrenaline steroid complexes, since according to the specificity test used by SHAW (1938) adrenaline yields a 2.5 times stronger colour reaction after addition of alkali than in acid solutions, but the reaction was only 1.06 times stronger in the blood (RAAB, 1943 d). These substances are adsorbed quantitatively on to aluminium hydroxide in pH 8.5 (RAAB, 1943 b). The same reaction is given by substances with a catechol nucleus (RAAB, 1943 b). RAAB found related substances in human arterial walls, kidneys (1943 a), heart muscle (1943 c, 1944), brain (RAAB, PEYSER and GIGEE, 1948) and in relatively high concentrations also in sympathetic ganglia (RAAB and HUMBREYS, 1947 a, b).

Methylene blue methods.

For the determination of adrenaline contents a combined biological and colorimetric method has also been used, where the rapidity of evanescence of the methylene blue colour under the influence of adrenaline is studied in the tissue mince (THUNBERG, 1918, AHLGREN, 1921, 1926, v. EULER and LILJESTRAND, 1929). According to v. EULER (1933 a), v. EULER and HOLMQVIST (1934) the adrenaline content of human blood is about 0.0001 μg %, less in ADDISON'S disease and more in fever (v. EULER, 1927). The same method was also used by several other workers (BROSS and KUBIKOWSKI, 1935, KONSCHEGG and MONAUNI, 1938, KOREFF and BENDEK, 1940). The former found increased values in endarteritis obliterans, low values in ADDISON's disease, and KONSCHEGG and MONAUNI (1938) increased values in nephritis. The method is nonspecific and the results can be affected by variations in the blood sugar contents (KONSCHEGG and MONAUNI, 1938). KOREFF and BENDEK (1940) obtained in the serum 0.2—0.02 μg % adrenaline and 0.4—0.04 μg % in the whole blood. The methylene blue method is sensitive, but the reading accuracy is low, as in biological methods.

Most chemical methods are nonspecific, because they are based on reactions of adrenaline in atom groups which are characteristic of many other substances. Thus it can be ascertained with the help of these reactions that perhaps some adrenaline is present but that the reaction can also have been caused by other substances. But even if the chemical methods are less sensitive and less specific than the biological ones they have nevertheless the advantage of being quantitatively more accurate.

B. Fluorescence methods.

Fluorescence methods are based on the oxidation of adrenaline in alkali. The first to dedect the appearance of a yellow-green fluorescence in adrenaline on addition of alkali was LOEW (1918). He found that adrenaline in stronger solutions was oxidised first to a labile red substance, in weaker solutions direct to a labile yellow substance, which gives fluorescence reaction. PAGET (1930) found that this reaction was specific for adrenaline. According to the researches of KONZETT and WEISS (1938, 1939) the fluorescence reaction is not given by substances even closely related to adrenaline, unless in very high concentrations. BARKER, EASTLAND and EVERS (1932) noticed the sensitivity of this reaction but they did not consider it appropriate for the quantitative determination of adrenaline. TIEGS (1934), KONZETT and WEISS (1939) recommended the fluorescence reaction also for the quantitative determination of adrenaline but considered it unreliable. GADDUM and SCHILD (1933) observed that in the light of an ultraviolet lamp, where the visible rays have been adsorbed with the help of a filter, the fluorescence reaction caused by 1 μg % adrenaline in an alkaline aqueous solution can still be seen with the naked eye. They were the first who endeavoured to adapt the fluorescence reaction to the determination of adrenaline in the peripheral blood, but found that the particular blue fluorescence of plasma and serum wholly concealed the greenish-yellow one of adrenaline, which fluorescence disappeared when proteins were precipitated with trichloracetic acid or metaphosphoric acid. This circumstance was observed also by TIEGS (1934), v. HUEBER (1940), LEHMANN and MICHAELIS (1942 a), JÖRGENSEN (1945) and v. PORAT (1946). GADDUM and SCHILD (1933) and JÖRGENSEN (1945) considered that adrenaline was adsorbed to the precipitated protein. v. PORAT (1946) has reported a method, where the protein precipitation is still in use.

Since the protein precipitation disturbs the determination of adrenaline in fluorescence methods, endeavours have been made to develop methods, where the adrenaline is dialysed from the blood against a weak acid solution (v. HUEBER, 1940, KALAJA and SAVOLAINEN, 1941 a, c, JÖRGENSEN, 1945, 1948, BLOCH, 1948) or where the adrenaline

is determined from plasma direct (LEHMANN and MICHAELIS, 1941, 1942 a, LEMAIRE, 1945).

v. HUEBER's (1940) reaction is chiefly qualitative. The first to use fluorescence reactions for the quantitative determination of adrenaline in the blood were KALAJA and SAVOLAINEN (1941 a, c). They found the normal adrenaline content in human blood to be 6—12 μg % (1941 b). JÖRGENSEN (1945, 1948) obtained by his method 4.1—9.6 μg %. WEST (1947 c) found in rabbit blood 9.8 μg % and in human blood 3—5 μg % adrenaline with JÖRGENSEN's method. v. PORAT (1946) did not confirm the above results, neither could he find any adrenaline in the blood by his own method. BLOCH (1948) did not find any adrenaline in the peripheral blood with JÖRGENSEN's method.

According to LEHMANN and MICHAELIS (1942 a, b, 1943 a, b) the adrenaline content of human plasma is on an average 186 μg %. In their subsequent researches STAUB and KLINGLER (1945) were unable to find any adrenaline with the help of the method of LEHMANN and MICHAELIS (1942 a), but considered the weakening of the adrenaline fluorescence reaction after the addition of formaldehyde used as a specificity test, to be due solely to the dilution of the solution.

EHRLÉN (1948 a) claims that adrenaline at first is oxidised under the influence of dissolved oxygen to adrenoquinone, which is stable in acid solution, but already at a pH of 2 there begins, through an intermolecular transposition, a fairly rapid transformation in a closed 5-ring, to leuco-adrenochrome and further to adrenochrome (fig. 1). By means of auto-

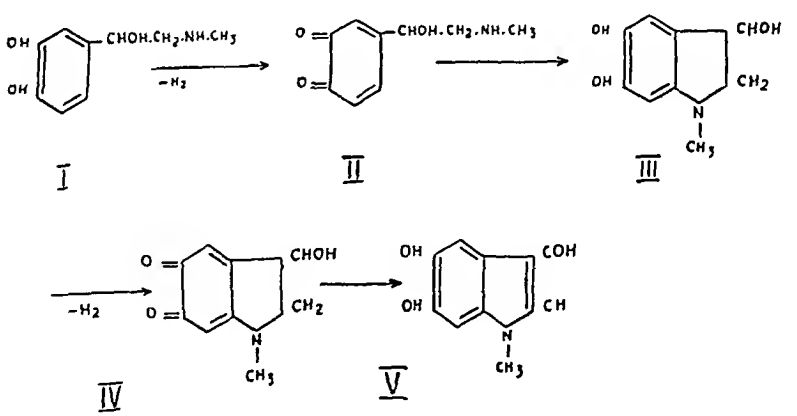

Fig. 1.

Oxidation of adrenaline to a fluorescent compound after addition of alkali. I. Adrenaline. II. Adrenoquinone. III. Leuco-adrenochrome. IV. Adrenochrome. V. "Fluorescent compound" or 1—CH3—3, 5, 6—trihydroxyindole.

reduction this can change to a fluorescing compound, 1-methyl—3, 5, 6-trihydroxyindole (EHRLÉN, 1948 a), exactly in the same way as in a melanisation process of tyrosine (RAPER, 1927). Formation of the fluorescent compound is not due to a reduction but to rearrangement of adrenochrome (EHRLÉN, 1948 a). In a caustic alkaline solution the red adrenochrome is immediately transformed into a yellow compound. Adrenochrome is oxidised already in a weak acid solution and the velocity of reaction increases in alkaline solution. EHRLÉN (1948 a) oxidises adrenaline first to adrenochrome, and by addition of a proper reducing agent he prevents a further oxidation of the fluorescent compound after an addition of alkali. The reducing agent will preserve the fluorescent compound from further oxidation by the dissolved oxygen. According to UTEVSKII (1944) and WEST (1947 a) leucoadrenochrome in ionic state causes the fluorescence reaction.

Ascorbic acid diminishes the fluorescence reaction of adrenaline, inhibiting it completely in concentration of ascorbic acid of 1.2×10.3 M. In concentration of less than $1.2 \times 10\text{-}4$ M ascorbic acid increases the fluorescence reaction of adrenaline. These effects are due to the reducing properties of ascorbic acid and its ability to act as a transmitter of hydrogen-ions (SUPEK, 1946). The fluorescence reaction of a solution of adrenaline varies linearly with a concentration according to SUPEK (1946), but not to EHRLÉN (1948 a).

Noradrenaline.

In this connection should be mentioned an adrenaline-like compound, noradrenaline, which gives the fluorescence reaction (fig. 2).

BARGER and DALE (1910) showed that the sympathetic effector substance might not be adrenaline but rather a catechol with a primary side chain, noradrenaline. CANNON and ROSENBLUETH (1933, 1935, 1937) observed more excitatory than inhibitory effects as compared with adrenaline during humoral transmission of certain sympathetic nerve stimulations. BACQ (1934) suggested that sympathin E is noradrenaline. It is now evident from the work of v. EULER that most mammalian tissues have comparatively high amounts of noradrenaline, catechol compounds, which differ in their effects from adrenaline and give only a very weak fluorescence reaction or none at all (v. EULER, 1946 a, b, c, 1947 a, b, 1948 a, b). They are found for instance in the sympathetic nerve trunk and adrenergic nerve fibres corresponding to adrenaline pressor equivalent of 3000—10000

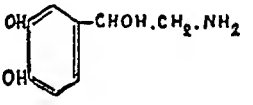

Fig. 2.
Noradrenaline or sympathin E.

TABLE 1.

CONTENT OF ADRENALINE IN THE BLOOD DETERMINED

A. BIOLOGICAL

Author	Method	Sensitivity μg %	Test object
BATTELLI, 1902 b	Blood pressure		Dog
GLEY and QUINQUAUD, 1913	"		Cat
POPIELSKI, 1911, 1916 a, b	"		Dog
SATO, 1932	"	100—1000	
EHRMANN, 1905, 1906, 1909	Pupilla	10—100	Cat
EICHLER, 1907	"		Man
KAUFMANN and MANNEBERG, 1907	"		Man
WIESEL and SCHUR, 1907, 1908	"		Man
GOLDZIEHER and MOLNAR, 1908	"		Man
WATERMANN and BODDAERT, 1908	"		Man
WATERMANN and SMITH, 1903	"		Rabbit
BORBERG, 1912	"	30	Man, rabbit
SCHNEIDER, 1922	"	100	Rabbit
BRANDT and KATZ, 1933 a, b	"	30—100	Man
KATZ, 1933	"	30—100	Man
BLOCK, 1933	"		
ROGOFF and MARCUS, 1938	"	0.1	Dog
SATO, 1932	"	10—100	
FRAENKEL, 1909	Uterus	5—100	Man
O' CONNOR, 1911 a, b, c	"	100—1000	Man, rabbit
FALTA and FLEMING, 1911	"		Dog
ADLER, 1914	"		Man, rabbit
TRENDELENBURG, 1910	Frog leg	0.1—5	Cat, rabbit
TRENDELENBURG and BRÖKING, 1911	"	10	Man, cat, rabbit
TRENDELENBURG, 1916, 1923 a	"	0.01—0.1	Rabbit
O' CONNOR, 1911 a, b, c,	"	10—70	Man, rabbit
KAHN, 1912 a, b	"		Dog, rabbit
NEGRIN Y LOPEZ, 1912	"		Rabbit
ROTHLIN, 1920	"	1	
HESS, 1922	"	0.001—0.1	Man
HÜLSE, 1922 a, b, c	"	0.001—0.1	Man
HÜLSE and VOLHARD, 1923	"		
v. EULER, 1926	"		Man
KURÉ, NAKAYA, MURAKAMI and OKINAKA 1932, 1933	"	10	Man

METHODS.

1 = peripheral vein
2 = artery
3 = upper part of inferior vena cava

Normal content μg %			Increased content μg %			Cause of increased content
1	2	3	1	2	3	
5—10						
	0					Stimulation of splanchnic nerve
0						After compression of aorta
0	0					
0			+			Nephritis
+			+			Nephritis
0	0		+			Anaesthesia, nephritis
0			+			Nephritis
0			0			Nephritis
0		+	+		+	Faradic stimulation of suprarenal glands
0	0					
		+				Strychnine
0	0		+	+		Hypoglycemia and shock
0	0		0	0		Muscle work
0	0					
0			0			Hypertonia
250			+			Nephritis, thyrotoxicosis
0	0	0				
0	0					
0			5—8			Thyrotoxicosis
50						
50	50					
0	0.05—0.1					
0						
0					+	Sugar puncture
0						
0	0		0	0		Nephritis, hypertonia, thyrotoxicosis
0	0		0	0		Asphyxia, hypertonia, nephritis
				+	2	Stimulation of splanchnic nerve
			2			Fever
50			100—160			Hypertonia
			70—80			Malignant renal arteriosclerosis

A. BIOLOGIGAL

Author	Method	Sensitivity μg %	Test object
WEST, 1947 b	Frog leg	10	Rabbit
SATO 1932	„	10—100	
HEILBRUNN and LIEBERT, 1939	„		Man
MEYER, 1906	Cow artery	0.1	Cat, rabbit
SCHLAYER, 1907, 1908	„		Man
ROTHLIN, 1920 a, b, c,	„	1—10	Cow, horse, rabbit
DEL CAMPO, 1918	*Rabbit ear*	*0.0001*	
ROTHLIN, 1920 a, b, c	„	1	
SCHLOSSMANN, 1927	„	0.0001—0.1	Cat, rabbit
SCHLOSSMANN and MÜGGE 1929	„		Cat
KAHLSON and v. WERZ, 1930	„	0.001—0.1	Man
HARTWICH and HESSEL, 1931 a, b	„	0.01	Rabbit
BRANDT and KATZ, 1933 a, b	„	0.0001—0.1	Man
KATZ, 1933	„	0.001—0.1	Man
MEYTHALER, 1935 a, b,	„		Man
MEYTHALER and WOSSIDLO, 1935	„		Man
OKAMURA, 1938	„		Man
SATO, 1932	„	1—10	
CANNON and HOSKINS, 1911	Intestine	0.2	Man
O' CONNOR, 1911 a, b, c	„		
STEWART and ROGOFF, 1924	„		
BARRE and HOUSSA, 1932	„		
BRANDT and KATZ, 1933 a, b	„	0.1—10	Man
KATZ, 1933	„	0.1—10	Man
OKAMURA, 1938	„		Man
SATO, 1932	„	4—80	

B. CHEMICAL

Author	Method	Sensitivity μg %	Test object
BATTELLI, 1902 a	Ferric chloride	3000	Dog
WIESEL and SCHUR, 1907	„		Man
STUBER, RUSSMANN and PROEBSTING, 1923	Iodate-sulfanilic acid-mercuric chloride	2	Man
BRANDT and KATZ, 1933 a, b			Man
CROCETTA, 1933	„		Man

METHODS (Continued).

Normal content μg %			Increased content μg %			Cause of increased content
1	2	3	1	2	3	
10						
50			+			Insulin
+						
			+			Nephritis
0						
0	0					
0	0			0.1—2		Operation, strychnine, sugar puncture
				0.05—0.2		Asphyxia, nicotine
0				0.04		Anaesthesia
0			0	0		Fever, hypertonia, thyrotoxicosis
0	0	+			2	Shock
0	0		+	+		Shock, hypoglycemia
0	0		0	0		Muscle work
+	+		+	+		Hypoglycemia
+	+		+	+		
0.5—2						
0			0			Asphyxia, sensory stimulation
0						
	0.1			0.7		Sensory stimulation
			+			Hypoglycemia and shock
0	0		+	+		Shock, sensory stimulation
0	0		0	0		Muscle work
0.5—2						

METHODS.

Normal content μg %			Increased content μg %			Cause of increased content
1	2	3	1	2	3	
+						
			+			Nephritis
50						
0	0		+	+		Hypoglycemia and shock
600						

B. CHEMICAL

Author	Method	Sensitivity μg %	Test object
DOGLIOTTI and CROCETTA, 1933	Iodate-sulfanilic		Man
VIALE, 1930, 1933 a, b	acid-mercuric	3	Man, dog
VIALE and CROCETTA, 1933	chloride		Dog
MACCHIARULO, 1935 a, b	„		Fetus
KONSCHEGG and MONAUNI, 1936	„		Man
KOBAYASHI, 1935	Phosphotungstic acid		Rabbit
WHITEHORN, 1935	Arsenomolybdic acid	2	Cat
DOPY and WEISINGER, 1938	„	0.2	Guinea pig
SARFY, 1938, 1939	„		Pigeon, guinea pig
SHAW, 1938	„	0.2	Man
	„		Rabbit
GIORDANO and ZEGLIO, 1939 a, b	„		Man
TIETZ, DORNHEGGEN and GOLDMAN, 1940	„	10	Man
BLOOR and BULLEN, 1941 a, b	„	0.1	Man, dog
RAAB, 1941, 1943	„		Man
KOBRO, 1936, 1946 a, b, c, d, e	„	1	Man
WEST, 1947 c	„	1	Rabbit
v. EULER, 1927	Methylene blue		Man
v. EULER, 1933 a	„	0.0000001	Man, rabbit
v. EULER and HOLMQUIST, 1934	„		Rabbit, hedgehog
BROSS and KUBIKOWSKI, 1935, 1936 ..	„		Man
KONSCHEGG and MONAUNI, 1938	„		Man
KOREFF and BENDEK, 1940	„		
v. HUEBER, 1940	Fluorescence		Man
KALAJA and SAVOLAINEN, 1941 a, b, c	„	1	Man
LEHMANN and MICHAELIS, 1942 a, b ..	„	4	Man, cat, dog, rabbit
JÖRGENSEN, 1945	„	1	Man
JÖRGENSEN, 1948	„	1	Rabbit
STAUB and KLINGLER, 1945	„		Man, rabbit
v. PORAT, 1946	„		Man
v. EULER and SCHMITERLÖW, 1947	„		Man, cow
WEST, 1947 c	„	1	Rabbit
BLOCH, 1948	„		Man

METHODS (Continued).

Normal content μg %			Increased content μg %			Cause of increased content
1	2	3	1	2	3	
250—330						
200—250						
70—250						
130—200						
50				+		Hypertonia
7	32					
0	2.5	17.5		4.5—6.5	24	Stimulation of sciatic or splanchnic nerve
0—0.4			2—10			Scorbut
2			3.4			Ascorbic acid treatment
			14			Ascorbic acid treatment after thyroidectomy
1.6—2						
5						
20—586						
0—120			100—200			Insulin
20—50						Adrenaline-like
6—22						Ascorbic acid and adrenaline
2.8—7.9						steroid-complex
10						
			0.2—0.01			Fever
0.0001			+—			More in fever, less in Mb.Addisoni
+						
0.003			+—			More in endarteritis obliterans, less in Mb.Addisoni
			+			Nephritis
0.02—0.4						
+						
6—12			12—30			Angina pectoris, bronchial asthma, fever, neurosis, thyro-
			12—40			Insulin toxicosis
71—468			0—1			Adrenalectomy
4—10						
7.4			3.7			Adrenalectomy
			17.4	11—12		Fright, asphyxia, stimulation of splanchnic nerve
0						
0						
0						
10						
0						

μg %, in the spleen 10000 μg %, in the heart, the liver and the muscles 300 μg %, in the blood only 4 μg %, in the brain little and in the placenta not at all (v. EULER, 1946 a, b, c, d, 1948 a, b, v. EULER and SCHMITERLÖW, 1947.

In recent years several investigators have conjectured that noradrenaline might be a sympathicomimetic substance in tissues and nerves (BACQ, 1933, 1934, 1935 a, b, 1947 a, b, BACQ and FREDERICQ, 1935, STEHLE and ELLSWORTH, 1937, GREER, PINKSTON, BAXTER and BRANNON, 1938, CRISMON and TAINTER, 1938, BACQ and FISCHER, 1947, v. EULER 1948 a, b, TAINTER, TULLAR and LUDUENA, 1948). Adrenergic nerve extracts contain a catechol derivate which differs chemically and pharmacologically from adrenaline and corresponds to noradrenaline (v. EULER, 1946 a, b, 1948 a, b). It is liberated by stimulating the sympathetic nerve. It is an amine in which, as compared to adrenaline, there is no methyl group of the side chain (fig. 2). It yields a very weak fluorescence reaction and colour; the fluorescence reaction is only 1/33 and the colour 1/13.5—1/16 in chemical tests as compared to adrenaline (v. EULER, 1946 a, WEST, 1947 b).

In certain tissues the substance extracted has the properties of adrenaline and not of noradrenaline. The sympathicomimetic substance extracted from the frog heart (LOEWI, 1936, v. EULER, 1946 c), prostata (v. EULER, 1934), postganglionic sympathetic nerve (LISSAK, 1938 b, 1939 a, b, CANNON and ROSENBLUETH ,1933, 1937, GADDUM and KWIATKOWSKI, 1938, 1939, GADDUM, YANG and KWIATKOWSKI, 1939), human coronary arteries and nerves (BACQ and FISCHER, 1947, BACQ, 1948), parotid gland of tropical toads, certain cells in the abdominal ganglion of annelides (GASKELL, 1939, BACQ, 1947 a) is adrenaline. The splenic nerves and sympathetic system of horse and calf contained both substances (BACQ and FISCHER, 1947, v. EULER, 1948 b).

Fairly consistent results have been obtained from suprarenal glands with significant amounts of adrenaline by chemical and biological methods (HATANO, 1936), about 1—2 times higher values by the latter, but in the blood to the contrary (table 1). The results obtained by chemical methods can be due to nonspecificity in the majority of methods.

Author's Researches.

1. Estimation of the fluorescence reaction of adrenaline.

The purpose of the following investigation is to give a closer analysis of the factors influencing the fluorescence reaction by a quantitative determination of adrenaline.

On addition of alkali to an aqueous solution of adrenaline there appears a greenish-yellow fluorescence (LOEW, 1918, PAGET, 1930, BARKER, EASTLAND and EVERS, 1932), which is quite visible in the light of an ultra-violet lamp, after the visible rays have been absorbed by a bluish-violet filter (GADDUM and SCHILD, 1933).

The control of the fluorescence is performed in a dark room. Before the colorimetry the investigator must adapt himself to the dark room for about 5—10 minutes and the ultra-violet lamp allowed to burn for about 10 minutes, when the lamp will have achieved the maximum of its light intensity. The reading has to be performed carefully as the correct estimation of a weak fluorescence is difficult, requiring some experience and observations of long duration. The fluorescent solution has to be placed as closely as possible to the source of light, preferably directly behind the colour filter, which facilitates the discrimination between small fluorescence intensities. The ultra-violet light area has to be homogeneous and the reading is always performed in the same spot of this field. The capability of the eye to discern the different intensities of the fluorescence diminishes in a state of fatigue.

The reading of the maximum of the fluorescence reaction is performed with the help of an eosin standard solution which KALAJA and SAVOLAINEN (1941 a, c) were the first to make use of and which later on has been employed also by JÖRGENSEN (1945, 1948) and PORAT (1946).

The fluorescence of the aqueous solution of eosin corresponds approximately in its colour to the fluorescence reaction of adrenaline solutions. The determination of adrenaline was performed with the help of an eosin test tube series (fig. 3) to which eosin had been added in such a way that the concentration of the previous tube surpasses by 10 % that of the following tube. A special frame (fig. 3) was prepared for the colorimetry, where the test tubes were placed at an angle of 45°. Every second place

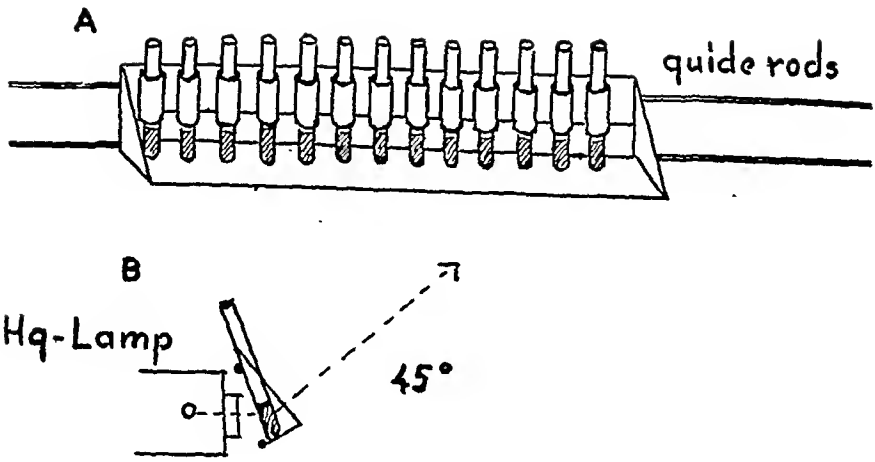

Fig. 3.
Frame with eosin test tubes. A = Front view. B = Side view.

was left empty, so that the test tube containing the adrenaline solution to be determined could be placed in it in order to perform the eosin comparison. The frame was placed on rails in such a way, that it could be turned transversely, and the test tube to be investigated could consequently always be placed in a suitable spot of the light field. Fig. 4 shows what adrenaline fluorescence reaction corresponded to the fluorescence of each eosin tube that has been used.

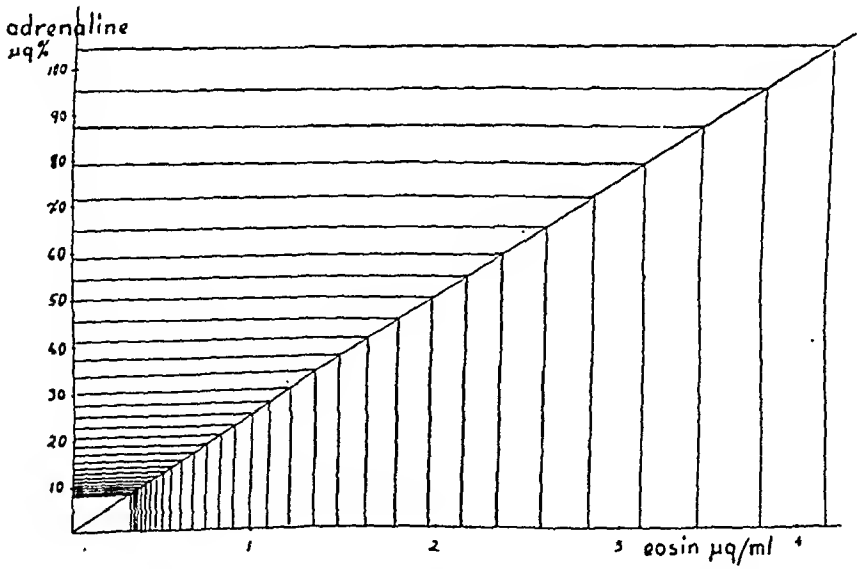

Fig. 4.
Eosin contents (µg/ml) corresponding to fluorescence reactions of known adrenaline solutions (µg %).

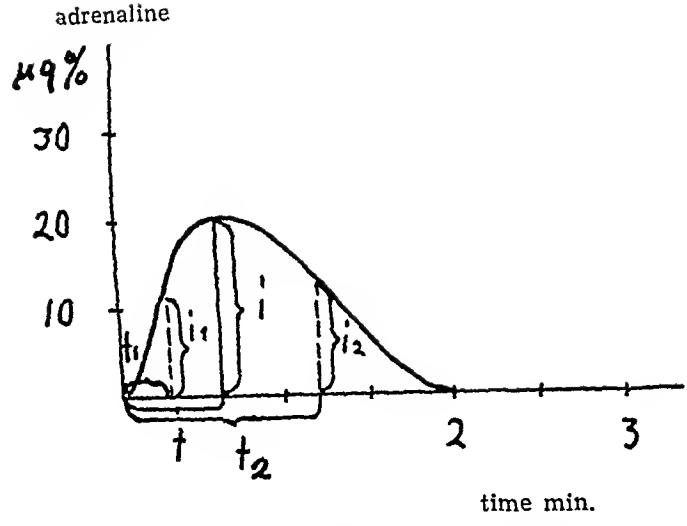

Fig. 5.

The fluorescence reaction of 20 µg % adrenaline solution. Time (t) corresponds to maximum intensity (i) of fluorescence reaction.

When determining the maximum fluorescence in adrenaline solutions, the duration (t) and the intensity of the reaction (i) must be taken into consideration (fig. 5). The fluorescence increases at first (i_1) soon attaining its peak and fading gradually (i_2). The maximum intensity appears usually in some seconds or minutes. The determination has to be performed when the peak is reached which, under the same testing conditions, is at the same point of time. By always observing the fluorescence reactions under the same special testing conditions we can use it also for a quantitative determination of adrenaline.

The eye can easily distinguish the fluorescence reaction of 1 µg % in pure aqueous solutions, but no smaller adrenaline contents. The demonstration of adrenaline in the recipient fluid is more difficult, because in dialysis methods the adrenaline of the blood is considerably diluted in the process of dialysis, in KALAJA and SAVOLAINEN's (1941 a, c) method 2.7-fold and in JÖRGENSEN's (1945) method 3.4-fold. The determination of adrenaline in the recipient fluid is disturbed by by-fluorescences. The diluting of adrenaline and the by-fluorescences of the blood put their natural limits to determinations of adrenaline in recipient fluid after dialysis from blood solutions.

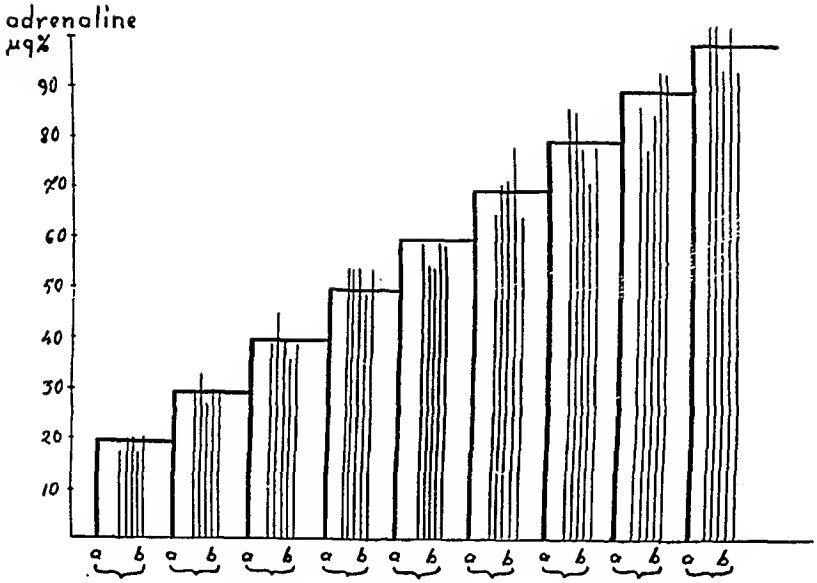

Fig. 6.
Determinations of the fluorescence reaction of 20—100 µg % adrenaline solution.
a = true adrenaline content
b = adrenaline content obtained by measuring with standard eosin solutions.

Pure distilled water does not fluoresce at all. The fluorescence brought about by the test tubes is also minimal, below 0.5 µg %. Even tubes manufactured from ordinary glass with thin walls of an even diameter can be used as test tubes. All tubes must have exactly the same inner diameter because even a small deviation suffices to produce considerable errors. Furthermore the fluorescence of the alkaline solution must be carefully checked, because a fluorescing solution might easily lead to erroneous deductions.

When measuring the fluorescence reaction of adrenaline the threshold of the eye which is about 1 : 10 must be taken into consideration. Consequently the accuracy of fluorescence determination cannot rise above 10 %. By performing fluorescence determinations with solutions the adrenaline contents of which are unknown to the investigator, it can be ascertained, that the limit of error remain below 15 % (fig. 6). When determining quite weak adrenaline contents of 1, 2, 3 µg % the error margin may rise to somewhat higher value (fig. 7).

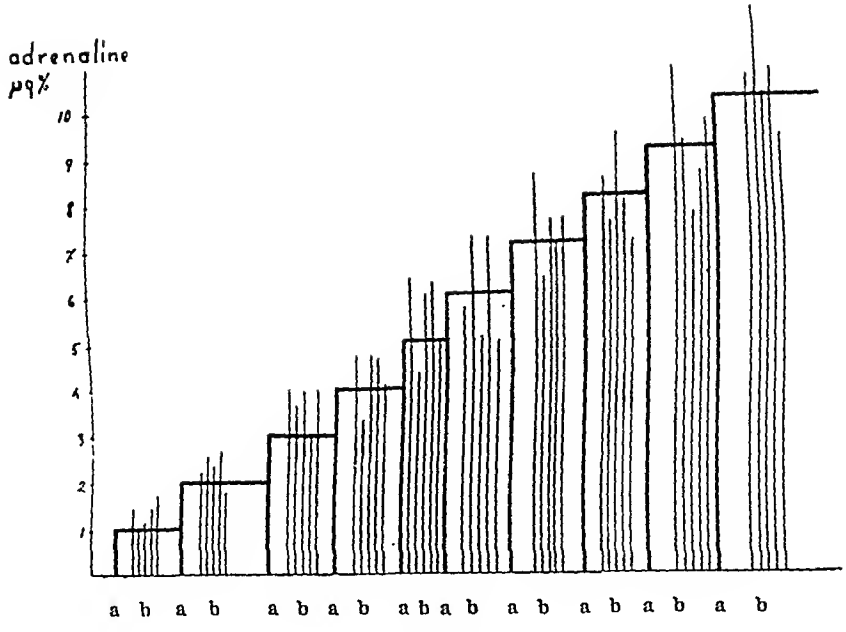

Fig. 7.
As above in fig. 6. 1—10 µg % adrenaline solutions.

2. Factors Influencing the Fluorescence Reaction of Adrenaline.

A. *Content of adrenaline.*

The maximum duration of the fluorescence reaction varies considerably depending on the amount of adrenaline. In small contents the maximum is of a shorter duration, but in large contents it has a considerably longer duration and makes a later appearance (fig. 8). Strong adrenaline concentrations are considerably more resistant to oxidising agents and heat than weak ones.

Several factors affect the development of the fluorescence reactions of adrenaline. GADDUM and SCHILD (1933) point out that for the formation of the fluorescence reaction of adrenaline not only alkali but also oxygen is needed. JÖRGENSEN (1945) has shown that in a nitrogen-atmosphere the adrenaline solution does not fluoresce at all, but it does fluoresce if oxygen is added to the solution. Neither does the adrenaline solution fluoresce if the oxygen is removed, for instance, with sodium hyposulphite (LEHMANN and MICHAELIS, 1942 a). The fluorescence of the adrenaline solution is influenced by the partial pressure of oxygen. This is a factor which must be taken into consideration when determining the adrenaline in plasma with the help of the fluorescence reaction, but it does not have any influence when measuring the adrenaline in a usual aqueous solution.

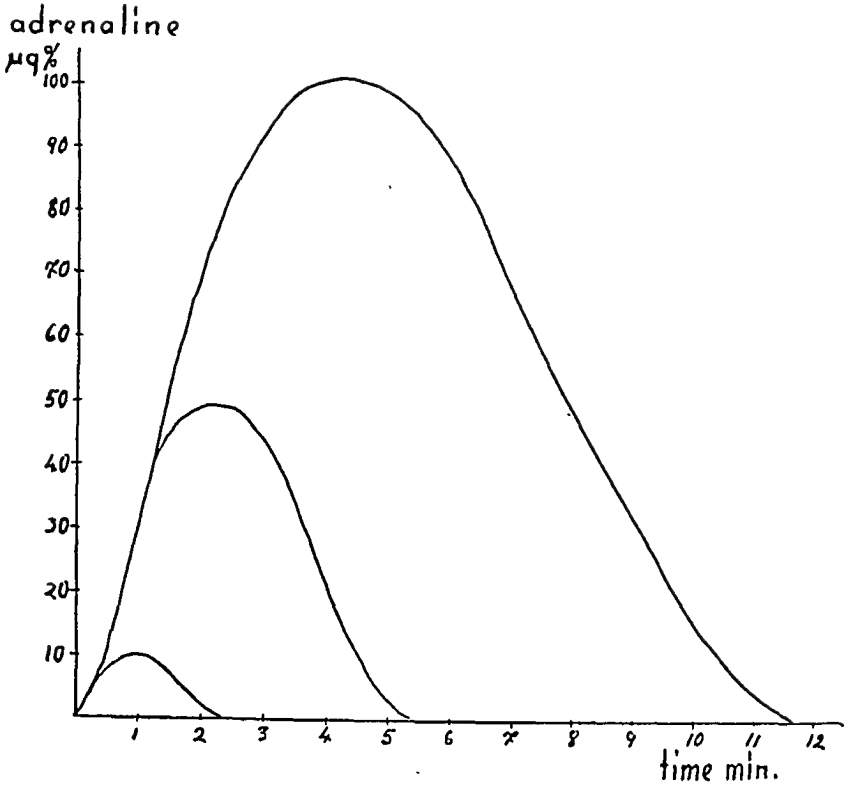

Fig. 8.
Effect of adrenaline content on intensity and duration of fluorescence reaction of 10,50 and 100 µg % adrenaline solutions.

B. *Oxidising agents.*

When alkali is added to strong adrenaline concentrations the first to become visible in daylight is a labile red colour which persists for some seconds, a similar colour as occurs when adrenaline is oxidised by an inorganic oxidising agent or enzymes. This red substance soon disappears and in its place a yellowish-green substance is formed, which slowly changes to a brown pigment, the definite oxidising product of adrenaline. At the yellow stage the adrenaline fluoresces. The yellowish colour persists but the fluorescence reaction disappears.

If the adrenaline is oxidised to a red compound with the help of v. EULER's iodine reaction (1933 b) or the orthophenolase of potatoes, this red oxidising product of adrenaline still fluoresces as strongly as the original adrenaline solution of a corresponding

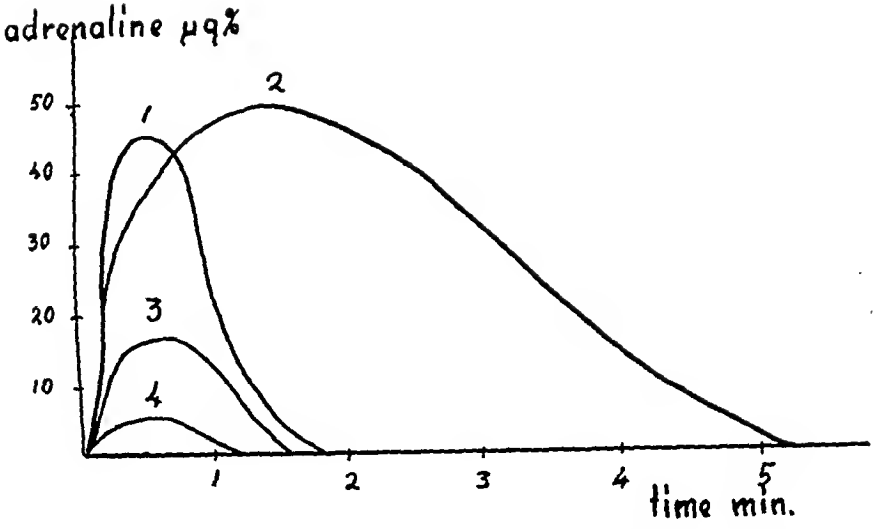

Fig. 9.
Effect of v. Euler's iodine reaction on the fluorescence reaction of 50 µg % adrenaline solutions.
1 = reaction after addition of sodium thiosulphate
2 = normal reaction
3 and 4 = reaction without sodium thiosulphate.

intensity (fig. 9). The only difference is that the reaction after oxidation is more rapid. Consequently the primary oxidising products of adrenaline also give this reaction. On the other hand, if adrenaline has been oxidised further than to its primary, red oxidation stage, the fluorescence reaction disappears. The results achieved in earlier investigations concerning the stability of adrenaline with the help of biological methods cannot therefore be compared to the results obtained by fluorescence methods, since the adrenaline molecule maintains its fluorescing qualities even at the first stage of oxidation, which is already biologically inactive. It is considered that in the inactivation of adrenaline two atoms of oxygen are needed for the first stage, when by the disappearance of four hydrogen atoms, adrenochrome is formed. The adrenochrome further forms melanin pigments. At this stage the fluorescence reaction of adrenaline also disappears.

However, the fluorescence reaction of the red oxidation product of adrenaline has a considerably greater velocity than of the usual aqueous solution of adrenaline (fig. 9) and also the oxidising agents together with alkali further catalyse it. In v. EULER's iodine

oxidation reaction (1933 b) the surplus iodine is removed with the help of sodium thiosulphate. If weak iodine is added as such as to the adrenaline solution without removing the surplus with thiosulphate, the surplus iodine so rapidly catalyses the oxidation of adrenaline on addition of alkali that the formation of a fluorescent intermediate product of adrenaline is impaired and the duration of the fluorescence reaction shortened. The addition of iodine can cause the oxidation of adrenaline to take place so swiftly, due to the influence of alkali, that the oxidation products of adrenaline give no fluorescence.

C. *Reducing agents.*

In contradistinction to iodine, which in alkaline circumstances is able to catalyse the oxidation of adrenaline to such a velocity that the fluorescent intermediate state is only quite insignificant, certain reducing agents can destroy this reaction protecting the adrenaline in acid or distilled water from oxidation. Of these substances the strongest is ascorbic acid. In these cases adrenaline does not produce any fluorescence reactions, since the reducing substances on the addition of alkali protect the adrenaline from being oxidised to the red adrenochrome, after which an addition of alkali can bring about the fluorescence reaction of adrenaline. An addition of 5 mg % of ascorbic acid to the adrenaline solution is enough to prevent the fluorescence reaction of adrenaline and even smaller additions of ascorbic acid have the power to render the formation of this reaction a great deal slower (fig. 10). Whilst the peak of the fluorescence reaction of adrenaline at 0.01 N hydrochloric acid solution appears within a minute, this maximum is not reached until after 4—5 minutes if ascorbic acid has been added. The weakening of this reaction proceeds in such cases equally slowly as its formation. The blood contains only 1—2 mg % ascorbic acid, and this quantity does not appreciably impair the determination of adrenaline in the dialysis method with the help of these reactions.

On the other hand, ascorbic acid is an important factor when determining the adrenaline in tissues containing it. It is interesting that fluorescence reaction is inhibited here

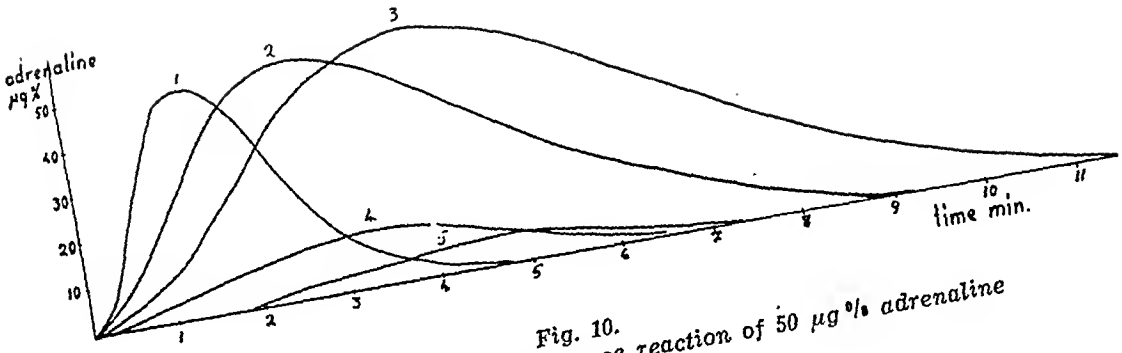

Fig. 10.

Effect of ascorbic acid on fluorescence reaction of 50 µg% adrenaline solution.

1 = reaction without ascorbic acid
2 = " in 1.25 mg% ascorbic acid solution
3 = " " 2.5 " " " "
4 = " " 5.0 " " " "
5 = " " 10.0 " " " "

by substances which safeguard the biological activity of adrenaline by protecting it from oxidation. The ability of the reducing substances to prevent the fluorescence reaction of adrenaline can be eliminated by oxidising them with iodine and removing the iodine surplus by means of sodium thiosulphate. The reaction is then catalysed equally rapidly as without addition of ascorbic acid (fig. 11).

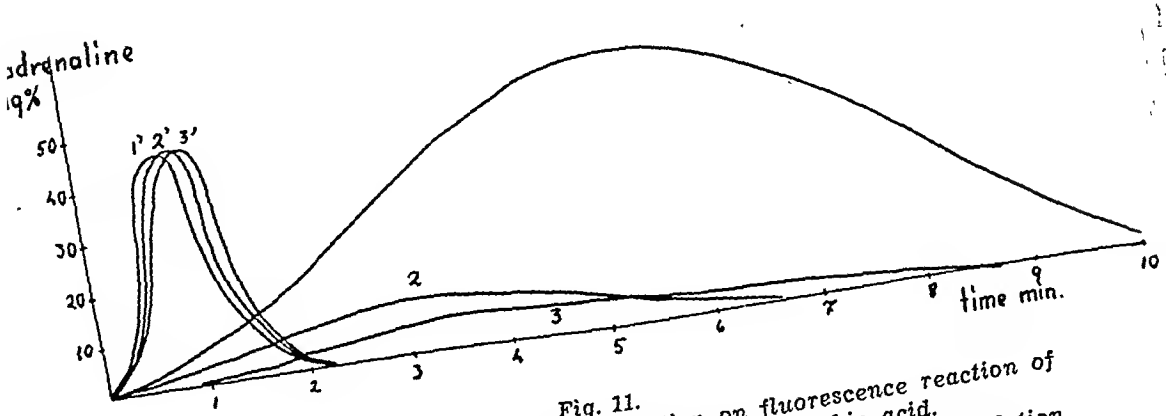

Fig. 11.

Effect of v. Euler's iodine oxidation reaction on fluorescence reaction of 50 µg% adrenaline solution containing ascorbic acid.

1 = 2.5 mg% ascorbic acid 1' = the same reaction after iodine oxidation
2 = 5.0 " " " 2' = " " " " "
3 = 10.0 " " " 3' = " " " " "

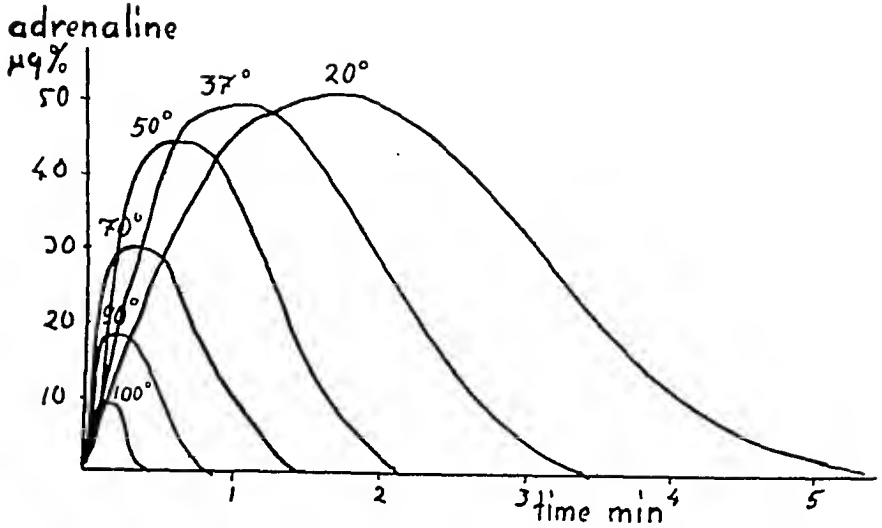

Fig. 12.
Effect of temperature on velocity and intensity of fluorescence reaction of 50 µg % adrenaline solution.

D. *Temperature.*

Temperature can destroy the fluorescence reaction of adrenaline (fig. 12). If the addition of alkali is performed at a certain temperature this catalyses the process of this reaction so as to render it more rapid. On the other hand it is possible to [illegible] solution to be investigated is cooled before the alkali is added (fig. 13). Even in an incubator at 37°C an aqueous solution of adrenaline maintains its fluorescence qualities quite well for 24 hours. v. PORAT (1946) has obtained the same results in his investigation concerning the preservability of adrenaline at room temperature. Adrenaline is preserved still better in a refrigerator than at a warm temperature.

E. *Acidity.*

The fluorescence reaction of adrenaline essentially depends also upon the quantity of alkali to be added to the adrenaline solution. As JÖRGENSEN (1945) has shown, the solution of adrenaline does not give any or only a very weak fluorescence reaction until the pH of the solution exceeds 11.

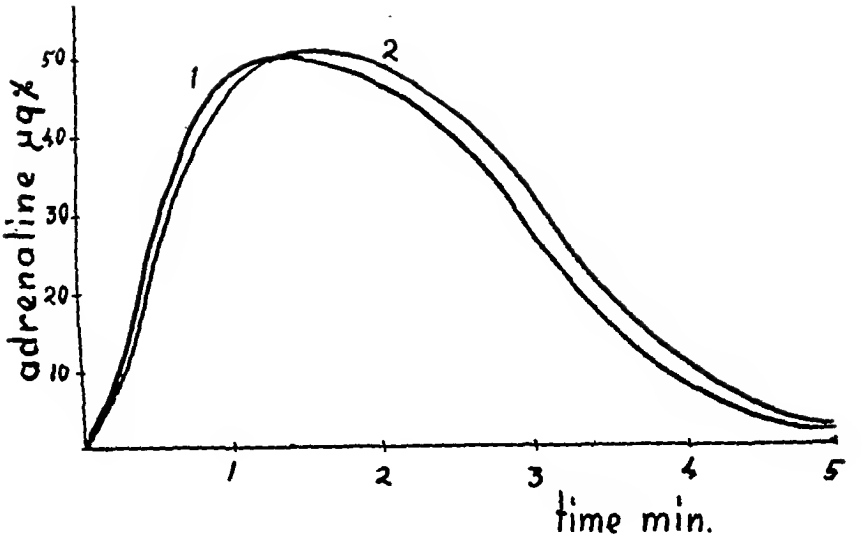

Fig. 13.
As above in fig. 12. 1 = Samples were kept for 10 min at 70° C, then cooled before addition of alkali.
2 = Normal reaction.

The acidity of the solution to be investigated also has its importance for the determination of the fluorescence reaction of adrenaline. Adrenaline still yields this reaction quite well in 0.01 and O.1 N hydrochloric acid if a sufficient quantity of alkali is added, but in 1 N hydrochloric acid the reaction weakens considerably (fig. 14). This phenomenon is to be found in all strong acids. This circumstance has its practical significance when attempts are made to determine the adrenaline from the blood solution after protein precipitation, because the acids used for precipitation, for instance trichloracetic acid and metaphosphoric acid, being fairly strong solutions of acid, destroy the fluorescence reaction of adrenaline. Consequently, the weak effects obtained are chiefly due to this circumstance and it is not necessary to seek the cause in an adsorption of adrenaline to the sediments of the precipitate, because as SHAW (1938) has demonstrated already the adrenaline remains in the solution at pH 4 and is best adsorbed at a pH above 7. Among others, the tungstic acid precipitation method used by v. PORAT (1946) proved to destroy the fluorescence of adrenaline. With this method only very weak

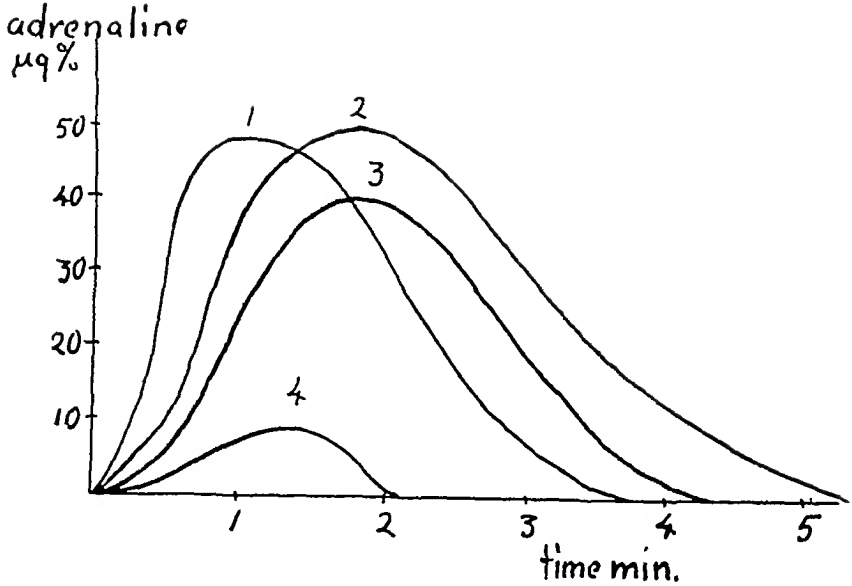

Fig. 14.
Effect of normality of hydrochloric acid on intensity of fluorescence reaction of 50 µg % adrenaline solution.
1 = reaction in aqueous solution
2 = „ in 0.01 N hydrochloric acid
3 = „ „ 0.1 „ „ „
4 = „ „ 1.0 „ „ „

reactions were achieved even on addition of large quantities of adrenaline to blood solutions.

There are also other substances which are able to destroy the fluorescence reaction of adrenaline, e.g. formaldehyde (CRAMER, 1911) and succinic acid (PAGET and LEBLOND, 1931, MARQUARDT 1938). Formaldehyde has also been used for specificity tests in VIALE's method (1933 a, b) in which adrenaline does not yield any colour if formaldehyde is added. The disappearance of the reaction with the help of formaldehyde is also parallel to the destruction of the biological activity of adrenaline (ABELOUS and DELAS, 1926, PAGET and LEBLOND, 1930, TOSCANO RICO and MALAFAYA BAPTISTA, 1935 a, MALAFAYA BAPTISTA, 1935, 1938). It is probable that the inactivation of adrenaline under the influence of formaldehyde is based on aldolic condensation (LEHMANN and MICHAELIS, 1942 a). JÖRGENSEN (1945) uses formaldehyde and succinic acid to destroy the fluorescence reaction of adrenaline in such a way that the concentration of these substances in an adrenaline solution are 1 and 0.4 %. The formaldehyde has an immediate effect. The succinic acid destroys the fluorescence reaction of adrenaline in about five minutes. According to the investigations of KONZETT and WEISS (1939) it is destroyed also by ultra-violet light.

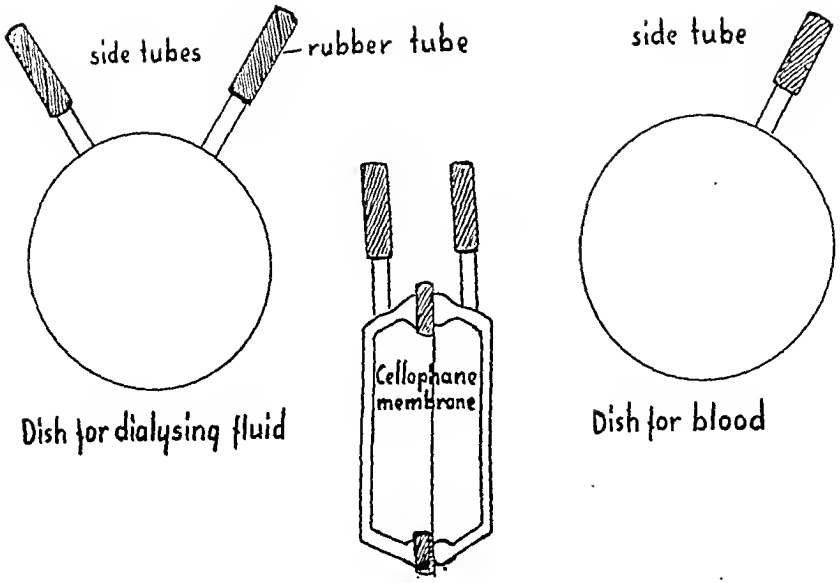

Fig. 15.
Structure of dialysing dishes.

3. The use of dialysis in adrenaline determination.

A. *Dialysis of adrenaline from aqueous solutions.*

The dialysis method for determination of adrenaline in the blood was adopted by v. HUEBER (1940). Adrenaline is dialysed from a hemolysed blood solution through a cellophane membrane into distilled water. The same method was also used by KALAJA and SAVOLAINEN (1941 a, b), JÖRGENSEN (1945, 1948), v. PORAT (1946) and BLOCH (1948). Prior to them STUBER, RUSSMANN and PROEBSTING (1923) and GIORDANO and ZEGLIO (1939) had already dialysed adrenaline from the blood, but they did not employ the fluorescence method for adrenaline determinations. Dialysis was carried out by them by means of pressure.

v. HUEBER (1940) used for dialysis the apparatus of DIETRICH described by LOEWI (1936) which has been used in these investigations also. It consists of two glass dishes with ground edges closely fitting together. Fig. 15 illustrates their cross-section and profile. The diameter of the dialysis vessel is about 4,5—5 cm and the depth 1 cm. 10 ml fluid fills about 2/3 of the capacity of one dialysing dish. The two dishes are separated by a cellophane membrane, through which the dialysis takes place. A thick piece of rub-

ber layer is placed between the edges of the dishes to prevent the fluid from trickling out. Fluorescent substances must not be derived from the rubber. One of the dishes has one and the other two side tubes. Their continuation is formed by rubber tubes about 4 cm long, which can be closed for the period of dialysis by a spring clip. The first dish is filled with the solution to be studied, and the other with dialysis fluid, which is easily poured out through the side tube on the completion of the dialysis process. Both halves of the dialysing vessel are held together by means of a rubber ring or a spring clip.

The dialysing dishes are carefully washed, because otherwise the by-fluorescences detaching themselves from them can disturb the determination of small quantities of adrenaline. The dishes are first washed in a mild alkaline solution and thereafter rinsed several times in distilled water so as not to leave any alkali in the dishes. In order to avoid the dishes breaking and the rubber hardening the drying is carried out in a moderate heat (30—40°C). To prevent chipping the edge of the dish is made somewhat thicker and rounded.

The cellophane membrane which is placed between the dishes is washed for several days before use in a weak acid and then distilled water, because the by-fluorescences originating from the cellophane may otherwise completely conceal the fluorescence reaction of the adrenaline under investigation. The fluorescence originating from the cellophane membrane may correspond to the fluorescence caused by an adrenaline solution of 10—30 μg %. After careful washing no fluorescent substances originate from the membrane. The cleanness can be tested by rinsing a small piece of cellophane in a little distilled water. If there is no fluorescence in this solution, the membrane is fit for use.

The dialysis is carried out in a shaker making about 120 horizontal movements per minute with an amplitude of 5 cm. When shaken in this apparatus, the adrenaline attains little by little its equilibrium on both sides of the membrane. A preliminary test can assure the persistence of the fluorescence reaction during the period of dialysis. Adrenaline solution is poured into some

test tubes and is allowed to stand for say six hours. The samples are compared with adrenaline solutions which have been dialysed for the same length of time. Adrenaline is preserved in both cases equally well.

It is a characteristic feature of dialysis methods that the cellophane membrane is easily permeable to adrenaline, but not to substances with large molecules such as proteins, nor does it adsorb adrenaline.

The dialysing properties of the cellophane membrane can be determined according to BJERRUM and MANEGOLD (1927, 1937) by measuring the thickness of the membrane when dry and saturated with water, the permeability for water and the capacity of the pores, and the difference in weight of the dry and the saturated membrane per unit of area and thickness. KALAJA and SAVOLAINEN (1941 a, b) and JÖRGENSEN (1945) used for adrenaline dialysis 17 mμ thick membranes. JÖRGENSEN (1945) has determined the above constants: thickness of the dry and of the wet membrane, its permeability to water and pore capacity.

These are, however, complicated measures. It is practical to make a direct test of the permeability of the membrane to adrenaline by using a water or hydrochloric acid solution containing adrenaline.

In order to determine the fitness of the membrane for dialysis an adrenaline solution of known concentration is introduced into one of the dishes by means of a pipette, and an equal amount of fluid free from adrenaline into the other dish. For these experiments 100 μg % aqueous and acid adrenaline solutions were used. Samples were taken at 15 to 30 minute's intervals during six hours. Fig. 16 illustrates the process of dialysis in different tests when using the same cellophane membrane. The dialysing begins at once and is half-done in an hour and a half. The adrenaline has gained its final equilibrium in three to four hours.

All membranes do not pass adrenaline equally well. Still, dialysis is completed within 4 to 6 hours with the majority of membranes. Fig. 17 illustrates the variations in dialysability between different membranes. The passability for dialysis is not determined by the thickness of the membrane alone, but above all by the size of its pores. It is to be remembered that not even the same membrane is in every respect homogeneous in its dialysing characteristics. It is therefore more advisable to use a

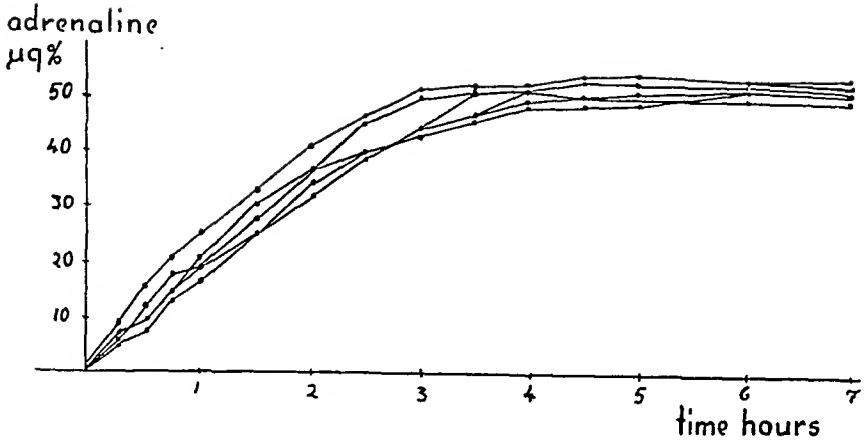

Fig. 16.

Dialysability of 100 μg% adrenaline through cellophane membrane from aqueous solution to aqueous solution. Content of adrenaline in the recipient fluid during dialysis.

dialysis time of 5 to 6 hours, for the equilibrium to be reached by adrenaline in a satisfactory manner. When using thin membranes the dialysis is somewhat more rapid than with thick ones. The pore diameter of thin membranes is, however, some-

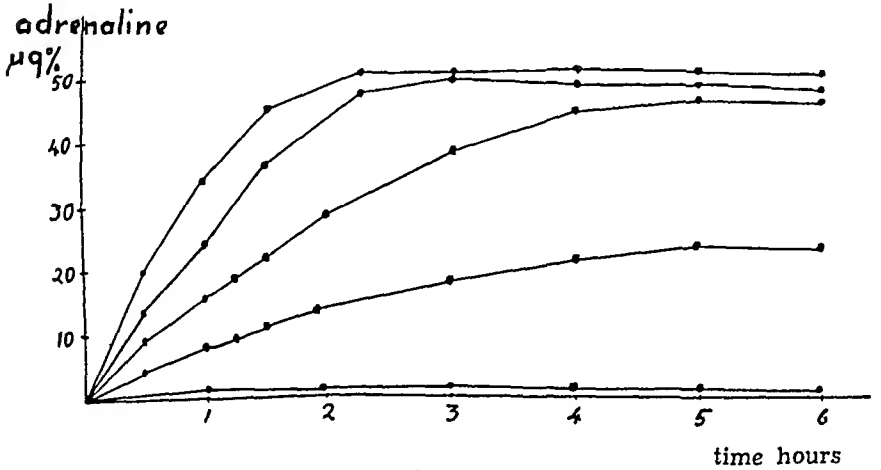

Fig. 17.

Dialysability of 100 μg% adrenaline from aqueous solution to aqueous solution using different cellophane membranes. Content of adrenaline in the recipient fluid during dialysis.

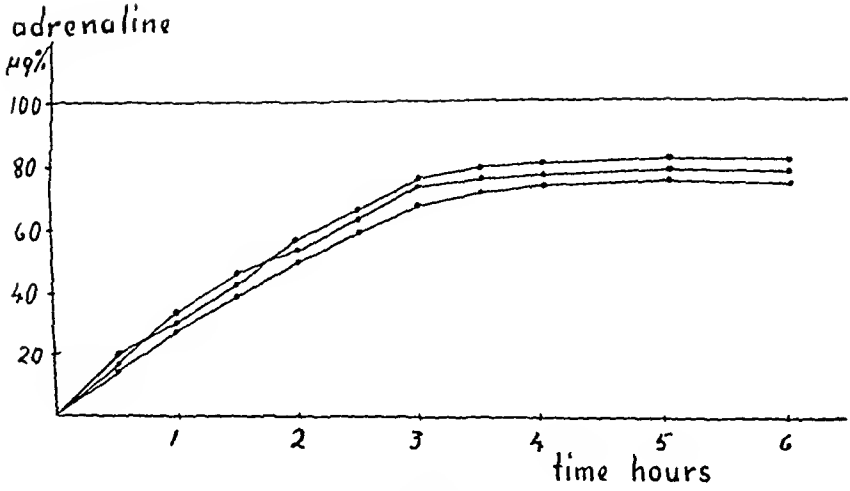

Fig. 18.
Dialysability of 200 μg % adrenaline from 0.01 N hydrochloric acid blood solution against a corresponding volume of 0.01 N hydrochloric acid. Content of adrenaline in the recipient fluid during dialysis.

times so small, that the adrenaline molecule cannot pass through it at all.

B. *Dialysis of adrenaline from blood solutions.*

The composition of the blood renders a determination of adrenaline by the dialysis method difficult. It may easily be supposed that adrenaline rapidly disappears from the blood.

LEHMANN, MICHAELIS (1942 a) and PORAT (1946) considered that adrenaline is not dialysed from the blood to equilibrium and disappears in the process of dialysis. To increase the preservability of adrenaline v. HUEBER (1940) added to the blood a few drops of hydrochloric acid. In order to stabilise the adrenaline KALAJA and SAVOLAINEN (1941 a, c) and JÖRGENSEN (1945, 1948) used an 0.01 N HCl solution with a further addition of 0.1 % glycocoll to heighten the stabilising effect. The blood is diluted by half with the same solution. KALAJA and SAVOLAINEN's (1941 a, c) blood solutions became hemolysed, but JÖRGENSEN's (1945) blood solution, to which citrated blood was added, remained partly free from hemolysis.

In these preliminary tests the blood of calf and cow has been used. In KALAJA and SAVOLAINEN's (1941 a, c) and JÖRGENSEN's (1945, 1948) blood solutions, to which adrenaline is added up to 200 μg %, the dialysis of adrenaline is studied by

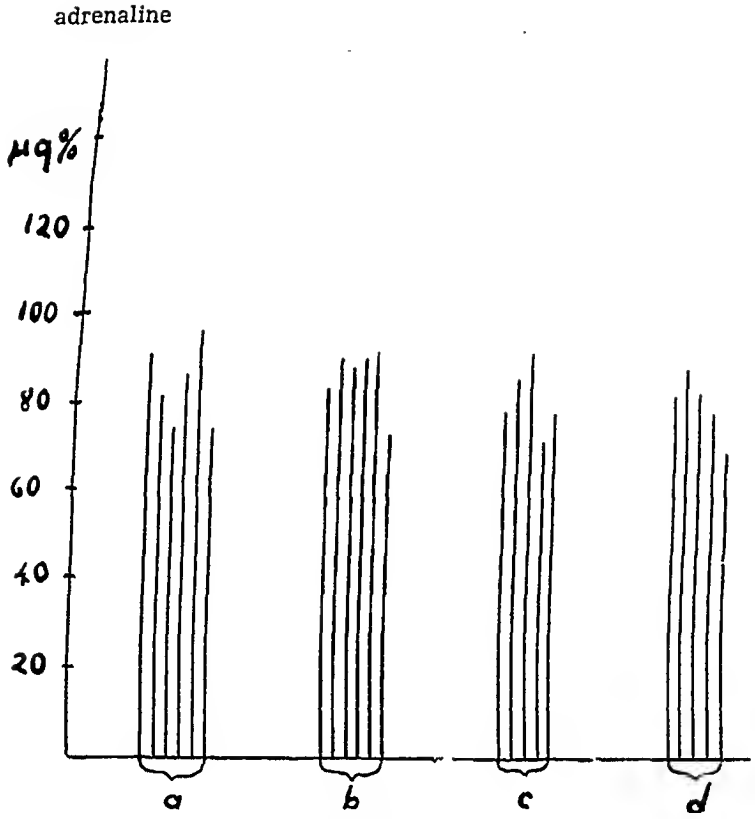

Fig. 19.
Preservability of 200 µg % adrenaline in hydrochloric acid glycocoll blood solution under dialysis and storing. a = content of adrenaline in recipient fluid immediately after dialysis. b = same after preserving adrenaline hydrochloric acid glycocoll blood 4 days in refrigerator. c = 4 days at room temperature. d = after 1.5 days in incubator at 37° C.

using as recipient fluid in the dialysis an equal volume of hydrochloric acid glycocoll solution (fig. 18). Adrenaline is dialysed to its final equilibrium after a shaking of four hours, but the dialysis is not complete as is in an aqueous solution. About 20 % of adrenaline is not dialysed. This is evidently partly due to the binding of adrenaline to protein substances and to DONNAN's membrane equilibrium. It is more reliable to use a dialysing time of five to six hours. Exactly the same results are obtained by using distilled water instead of the hydrochloric acid glycocoll solution as diluent of blood and as recipient fluid.

An organic base, such as adrenaline, can be combined also with protein (BÜTTNER, 1933, UTEVSKII, 1936, 1938, 1939, UTEVSKII and PESSKINA, 1939).

Adrenaline is easily dialysed and retains its fluorescent characteristics quite well during the period of dialysis when the dialysis is carried out from a blood solution. Even if the samples are in the shaker for a period of 24 hours, adrenaline does not evanesce.

Adrenaline can be preserved for 4 days in a refrigerator when up to 20—200 μg % of it are added to a hydrochloric acid glycocoll blood solution and hydrochloric acid glycocoll citrated blood solution (fig. 19). At room temperature adrenaline is also preserved in blood solution for severals day's time. In an incubator at 37°C adrenaline retains is fluorescence ability for a day and a half. On the other hand, if stored for a longer time at 37°C in a blood solution, the fluorescing capacity of adrenaline is weakened and autolysing agents destroy the proteins in the blood. The blood stabilises the fluorescence reaction characteristic of adrenaline quite well even in those cases where no stabilising substances have been added to the blood, as proved by the results obtained with citrated blood.

If known quantities of hydrochloric acid or sodium hydroxide are added to the blood solution, in such a way that the pH of the blood varies between 3—10, it may be observed that adrenaline is recovered in about equal strenghts at pH of 4—8.5 (fig. 20), but to a smaller extent, however, in more basic and more acid ranges. In an alkaline range at a pH exceeding 9 it seems that adrenaline begins to become oxidised under the influence of alkali during the period of dialysis, and gradually loses its fluorescing characteristics, in the same way as when strong alkali is added to the adrenaline solution, after which the fluorescence reaction quickly disappears. In an acid range with a pH below 4 the denatured proteins of the blood, a thick and tarlike mass, render the dialysis of adrenaline impossible. Dialysis can well be carried out for instance from sodium citrate blood as such direct against distilled water.

C. *Experiments with dialysis methods.*

KALAJA and SAVOLAINEN (1941 a, c) used the following method for determining adrenaline in the blood solution: a sample of 10 ml blood and 10 ml stabilising solution are mixed together in a test tube until the blood becomes hemolysed and its coagulation prevented. The stabilising

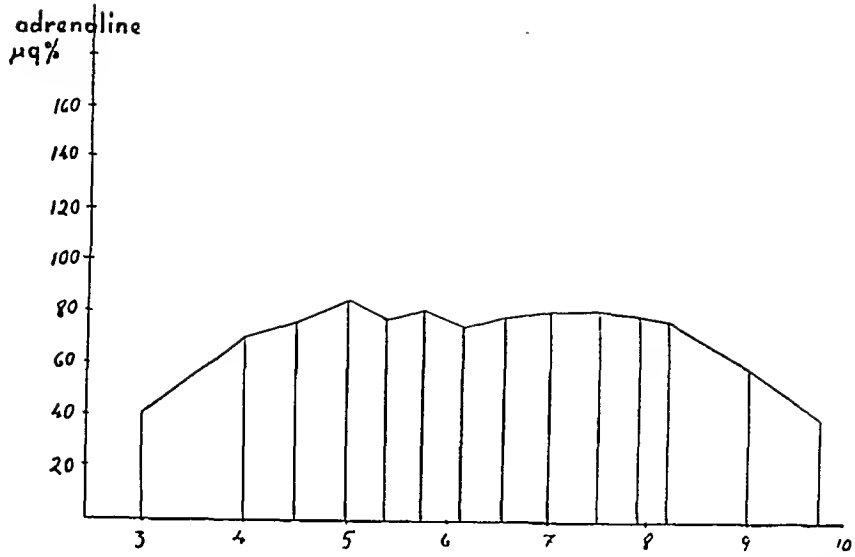

Fig. 20.

Dialysability of 200 µg % adrenaline from blood solutions at different pH-ranges. Content of adrenaline in the recipient fluid after dialysis.

fluid consists of 0.01 N hydrochloric acid solution, to which has been added 0.1 % glycocoll. 10 ml of the diluted blood is pipetted into one of the glass cups of the dialysis apparatus. The corresponding other half is filled with 2.5 ml hydrochloric acid-glycocoll solution to which has been added 1 ml of a suspension of aluminium hydroxide. The purpose of the aluminium hydroxide is to eliminate disturbing by-fluorescences. The aluminium hydroxide is prepared according to SHAW (1938). The dialysis is carried out in a shaker for 4—4½ hours, whereafter the adrenaline is determined in the centrifuged filtrate by means of the fluorescence reaction in ultra-violet light. For this purpose 2 ml clear dialysate are pipetted into a test tube of even thickness with a diameter of 1 cm. In order to bring about the fluorescence reaction, 0.3 ml 5 N sodium hydroxide is added to the solution. The fluorescence reaction then appearing is measured by comparing it to a known eosin solution. 2.3 ml distilled water in a test tube is mixed with the eosin solution added to it from a microburette, until the fluorescence of this dilution attains the same intensity as the fluorescence reaction of the adrenaline solution under examination. If the amount of adrenaline in the dialysing fluid is known, it is easy to estimate the adrenaline content of whole blood. The result is given in µg %.

JÖRGENSEN's (1945, 1948) method of adrenaline determination is a modification of the former. 10 ml venous blood is withdrawn into a test tube into which 0.5 ml saturated sodium citrate solution has been pipetted. The water is evaporated from the sodium citrate before adding

the blood specimen. The dilution of the blood is carried out in exactly the same way as described by KALAJA and SAVOLAINEN (1941 a, c). The dialysis is carried out against a recipient fluid containing glycocoll hydrochloric acid and an aluminium hydroxide suspension twice as large as in KALAJA and SAVOLAINEN's (1941 a, c) method, to enable one to demonstrate adrenaline in two parallel samples, the determination of the fluorescence reaction in one sample being more difficult. In order to separate the particles of aluminium hydroxide from the dialysing fluid containing adrenaline, JÖRGENSEN (1945, 1948) carries out a centrifugation at a rate of 8000 revolutions per minute. The determination of the fluorescence reaction in the centrifuged solution is carried out with the help of an eosin standard solution as in KALAJA and SAVOLAINEN's (1941 a, c) method, but less alkali is added, so that the fluorescence reaction reaches its highest intensity within a minute, and has a longer duration than if stronger alkali is used. 0.3 ml 2 N sodium hydroxide is added to the clear dialysate. The adrenaline content is obtained direct from a standard curve, where the eosin consumption is ordinate, and the adrenaline content abscissa.

If adrenaline is added to ordinary blood or citrated blood which has been diluted in the above-described way, it can be found that results obtained from the recipient fluid containing aluminium hydroxide are considerably weaker than the expected results. Fig 21 illustrates the results obtained on addition of 100 and 300 μg % adrenaline to the blood. Exactly the same phenomenon can be observed when adding smaller quantities of adrenaline. When using large additions of adrenaline the results demonstrate more clearly, to what extent adrenaline is recovered after dialysis. Only 6—10 % of the original values of adrenaline are recovered from citrated blood. When using ordinary blood the results are somewhat better, but varying. In part of the samples it is possible to recover adrenaline in quite a satisfactory manner, but in some cases none at all.

Since in the dialysis tests without aluminium hydroxide adrenaline added to the blood could very well be demonstrated in the recipient fluid after dialysis, but considerably less well if aluminium hydroxide was added to the dialysing fluid, it becomes evident that aluminium hydroxide has played its part in the weakening of the effects obtained. It can then either be supposed that aluminium hydroxide weakens the fluorescence reaction of adrenaline,or that adrenaline has become adsorbed to the alumin-

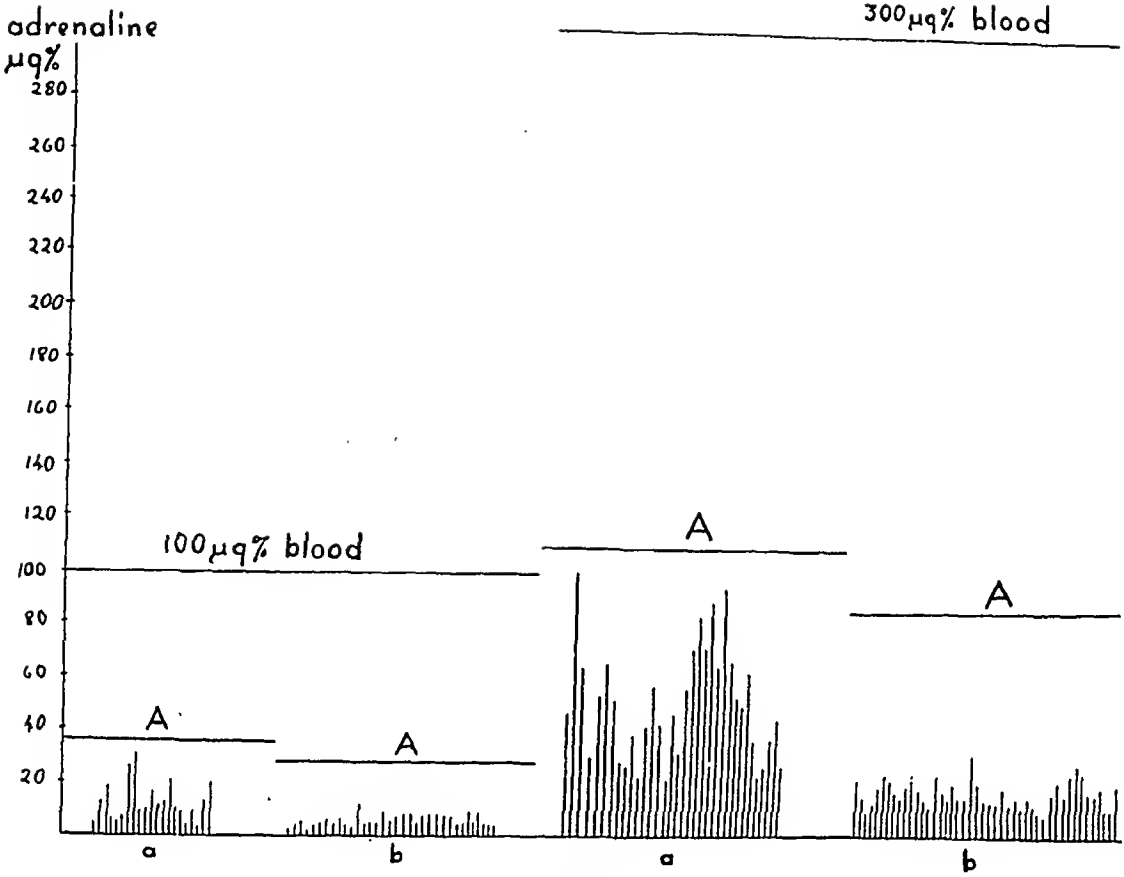

Fig. 21.
Dialysability of adrenaline in the filtrate after dialysis from hydrochloric acid ordinary (a) and citrated (b) blood solutions. 100 and 300 µg % adrenaline added to the blood. A = expected content of adrenaline in the filtrate if results were quantitative.

ium hydroxide, after which the centrifuged filtrate becomes free from adrenaline.

The following tests tend to prove that aluminium hydroxide as such need not weaken the fluorescence reaction of adrenaline (fig. 22). On addition of alkali to a suspension of aluminium hydroxide in hydrochloric acid or distilled water, the particles of aluminium hydroxide are dissolved, and the blue fluorescence due to these particles disappears, the solution remaining as free from fluorescence as without aluminium hydroxide, providing that the suspension of the latter is prepared with care, excluding from it disturbing by-fluorescences. This test can also be used for determinations of the usability of aluminium hydroxide. When

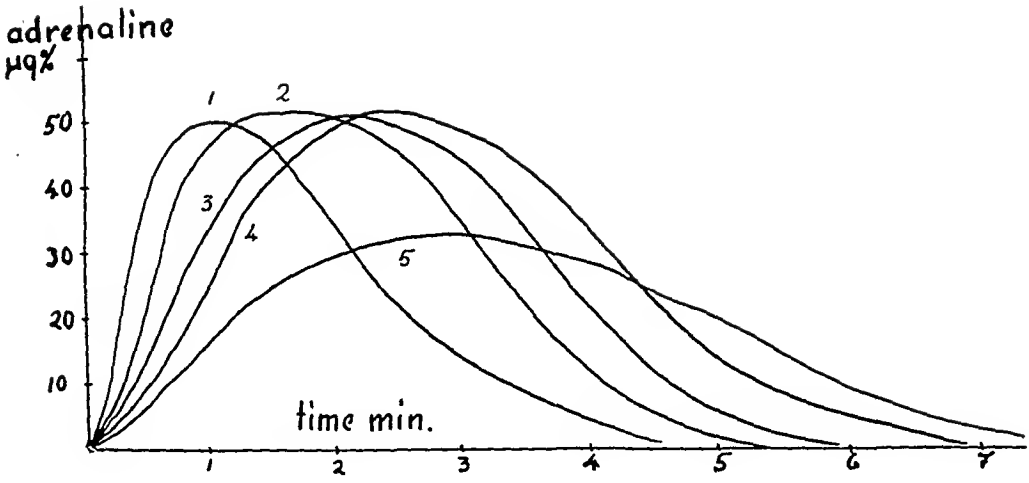

Fig. 22.
Effect of aluminium hydroxide on the fluorescence reaction of 50 µg % adrenaline solution.

1 = 2.0 ml 0.01 N HCl
2 = 1.75 „ „ „ „ and 0.25 ml aluminium hydroxide suspension
3 = 1.5 „ „ „ „ „ 0.5 „ „ „ „
4 = 1.25 „ „ „ „ „ 0.75 „ „ „ „
5 = 1.0 „ „ „ „ „ 1.0 „ „ „ „

making tests where there is also adrenaline in the solution and parallel tests where adrenaline has been added to distilled water, it is possible to find in both solutions, on addition of alkali, equally strong fluorescence reactions, even if the reaction in the distilled water has a greater velocity than in the solution containing aluminium hydroxide. When using larger amounts of aluminium hydroxide the fluorescence reaction of adrenaline somewhat weakens. The colour of the fluorescence reaction is also somewhat paler than the yellowish-green fluorescence appearing in aqueous adrenaline solutions after the addition of alkali. In some rare cases a lower concentration of aluminium hydroxide can also weaken the fluorescence reaction.

In order to elucidate the adsorption of adrenaline in the sediment of aluminium hydroxide, an adrenaline determination is carried out after dialysis not only in the centrifuged filtrate, but also in the aluminium hydroxide sediment which is mixed with 1 ml distilled water. The experiments show that when using a hydrochloric acid glycocoll citrated blood solution, the

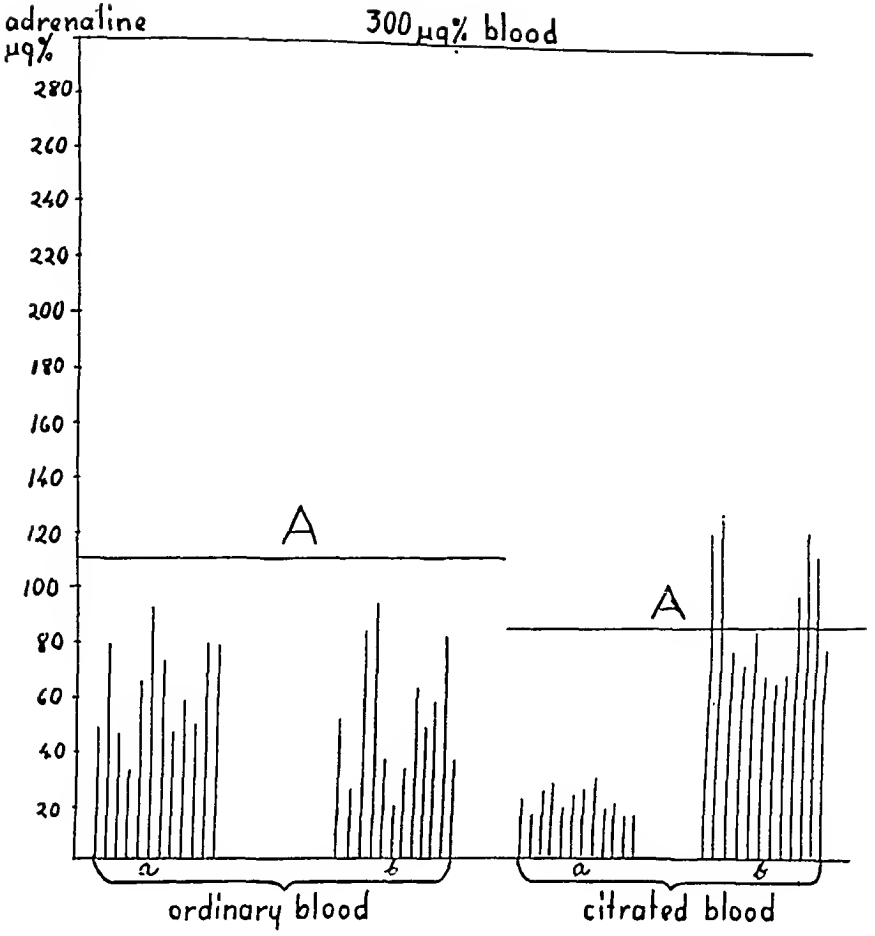

Fig. 23.

Adsorption of adrenaline to aluminium hydroxide after dialysis from hydrochloric acid ordinary and citrated blood solutions. 300 μg% adrenaline added to the blood.

a = content of adrenaline in the filtrate
b = „ „ „ „ „ aluminium hydroxide precipitate
A = expected content of adrenaline in the filtrate.

adrenaline can be determined after dialysis nearly quantitatively in the aluminium hydroxide sediment, whereas there is only very little adrenaline in the corresponding filtrate. The results obtained are illustrated by fig. 23. During the dialysis the adrenaline has thus become adsorbed to the aluminium hydroxide.

When determining the pH of ordinary and citrated blood diluted by half with the 0.01 N hydrochloric acid glycocoll solution it is found that pH of the former is 7.1—7.2, and of the

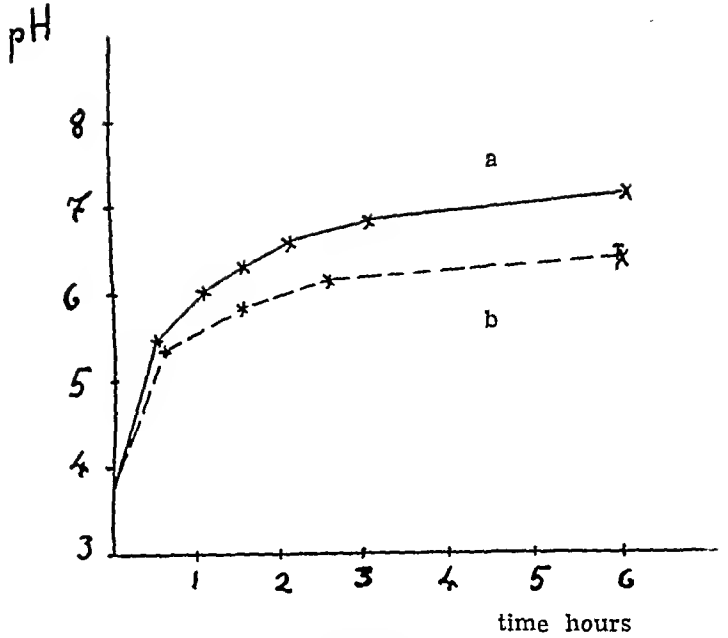

Fig. 24.

Changes of pH in the recipient fluid during dialysis of adrenaline from hydrochloric acid ordinary (a) and citrated (b) blood solutions.

latter 7.2—7.3. The undiluted ordinary blood has a pH 7.3—7.4, and undiluted citrated blood a pH of 7.5.

Thus the 0.01 N hydrochloric acid glycocoll solution used as diluting fluid of the blood reduces the pH by 0.2—0.3 units only. If 2.5 ml hydrochloric acid glycocoll solution is used as recipient fluid together with 1 ml aluminium hydroxide the suspension has a pH of 4. When dialysing adrenaline from citrated blood dilution the pH of the recipient fluid rises after a four hour's dialysis to 7.0. During dialysis from an ordinary blood dilution the pH of the recipient fluid rises from 4 to 5.5 already within half an hour and to 6.2 after four hours (fig. 24). Thus the recipient fluid does not retain its original pH, at which adrenaline remains in the solution, but rises rapidly causing thereby a partial or complete adsorption of adrenaline to the aluminium hydroxide. If adrenaline is dialysed from citrated blood dilution the pH of the recipient fluid rises higher than when dialysed from an ordinary blood dilution. It is therefore evident that adrenaline

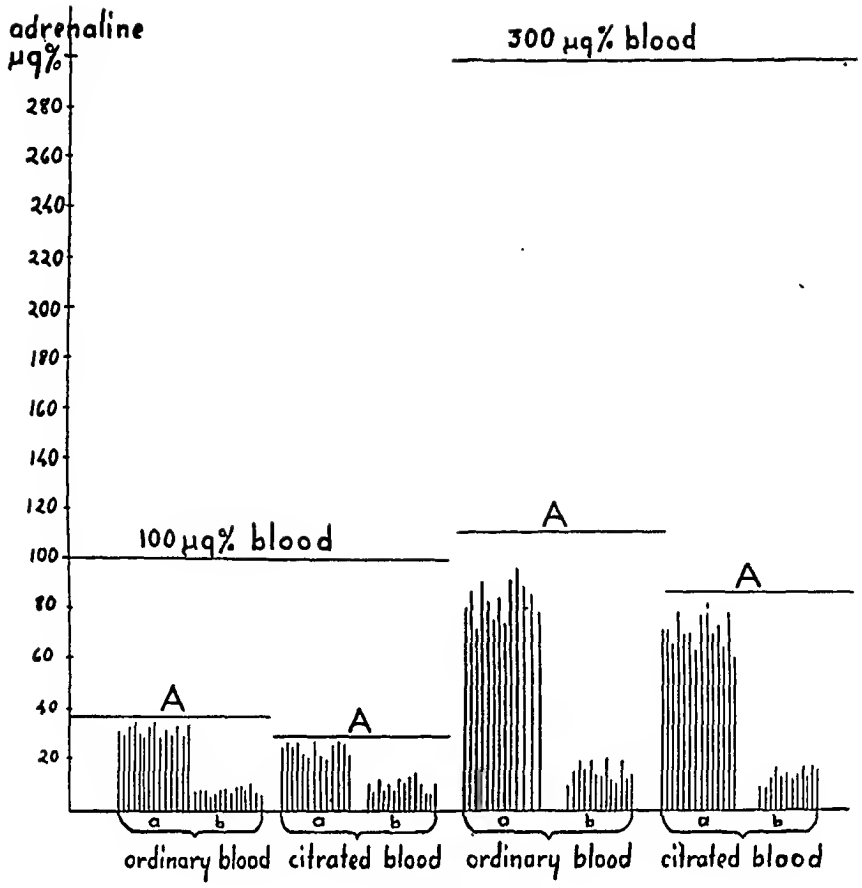

Fig. 25.

Adsorption of adrenaline to aluminium hydroxide during dialysis when the pH of the recipient fluid has been increased to 7.5—8 by addition of sodium hydroxide in the hydrochloric acid ordinary and citrated blood solutions. 100 and 300 µg % adrenaline added to the blood.

a = content of adrenaline in the precipitate

b = " " " " " filtrate.

A = expected content of adrenaline in the filtrate.

is adsorbed to the aluminium hydroxide sediment especially in the former case whereas, in the latter, part of the adrenaline can remain in the solution. Clearly the pH of the latter solution is close to that border where adrenaline is either absorbed to the aluminium hydroxide or persists in the solution. Adrenaline is adsorbed fairly completely to aluminium hydroxide precipitate, when the pH of the dialysing fluid has been increased to 7.5—8 by addition of sodium hydroxide in the blood dilution (fig. 25).

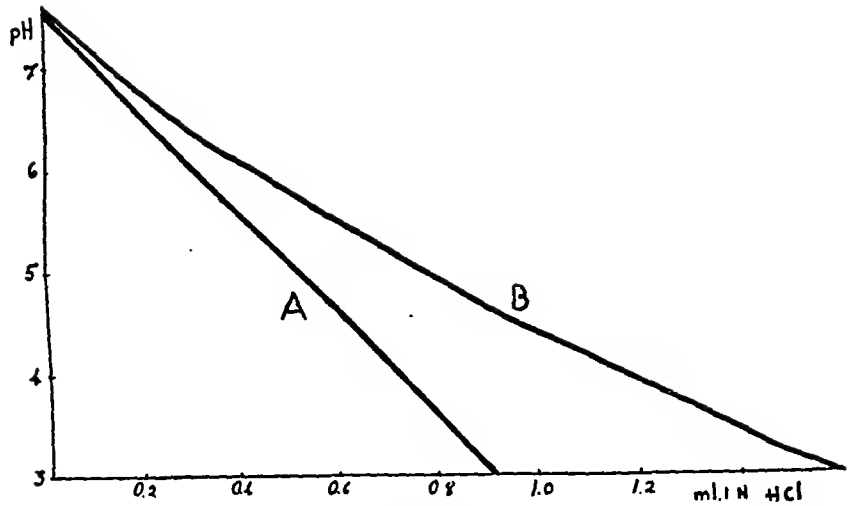

Fig. 26.
Changes of pH after addition of 1 N hydrochloric acid to 10 ml of ordinary (A) and citrated blood (B) solutions diluted by half with distilled water.

D. *Author's modification of dialysis methods.*

Since adrenaline is adsorbed to aluminium hydroxide in the dialysis methods described above, attempts have been made to develop methods where this adsorption to aluminium hydroxide would be prevented. In order to maintain the pH of the dialysing fluid closer to pH 4 a stronger concentration of hydrochloric acid must be used for diluting the blood. Fig. 26 illustrates the effect of an addition of 1 N hydrochloric acid on the pH of the blood solution. The pH of the diluted blood is the ordinate, and the addition of hydrochloric acid the abscissa. The curves illustrate the changes in pH of ordinary and citrated blood dilutions. If the blood is diluted by half with 0.1 N hydrochloric acid solution the pH of the ordinary blood dilution decreases to 5.2 whereas the pH of the citrated blood dilution falls to pH 5.7.

In order to prevent the adsorption of adrenaline to aluminium hydroxide, instead of a 0.01 N hydrochloric acid 0.07 N hydrochloric acid has to be used as diluent for ordinary and 0.1 N hydrochloric acid for citrated blood. The blood dilutions then have a pH of about 5.7. After the process of dialysis the pH of the recipient fluid thus remains at between 4—5.

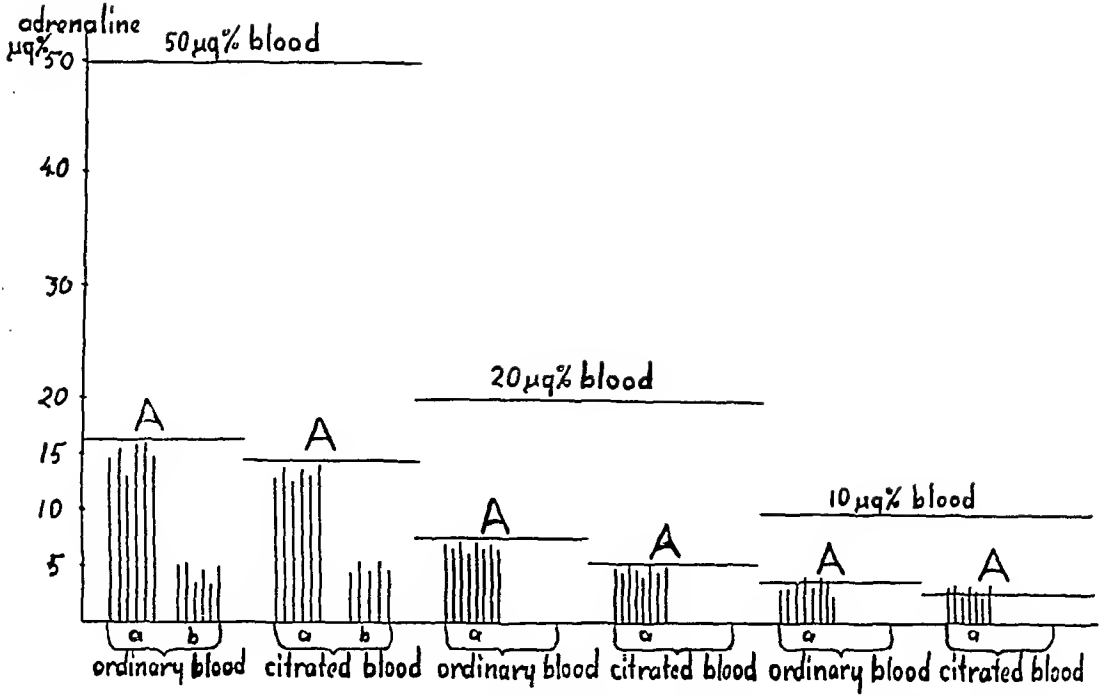

Fig. 27.
Content of adrenaline in the filtrate (a) and in the aluminium hydroxide precipitate (b) after dialysis from hydrochloric acid blood solution when the ordinary blood has been diluted by half with 0.07 N and the citrated blood by half with 0.1 N hydrochloric acid.
10,20 and 50 µg % adrenaline added to the blood
A = expected content of adrenaline in the filtrate.

When using the above stronger hydrochloric acid as diluting fluids, it is observed that adrenaline added to the blood is recovered from the recipient fluid after completed dialysis in the expected amounts (fig. 27 and 28). Experiments have been carried out with adrenaline solutions of 10, 20, 50, 100 and 300 µg %. When determining adrenaline from the aluminium hydroxide sediment in these experiments, only slight fluorescent effects can be observed, which shows that adrenaline has not become adsorbed to the aluminium hydroxide, but has remained in the filtrate.

By altering the pH in blood dilutions from 3.5 to 8.5 at intervals of half a degree by additions of hydrochloric acid and sodium hydroxide solutions, it is possible to demonstrate over what pH-range the adrenaline is retained in the solution or adsorbed to the

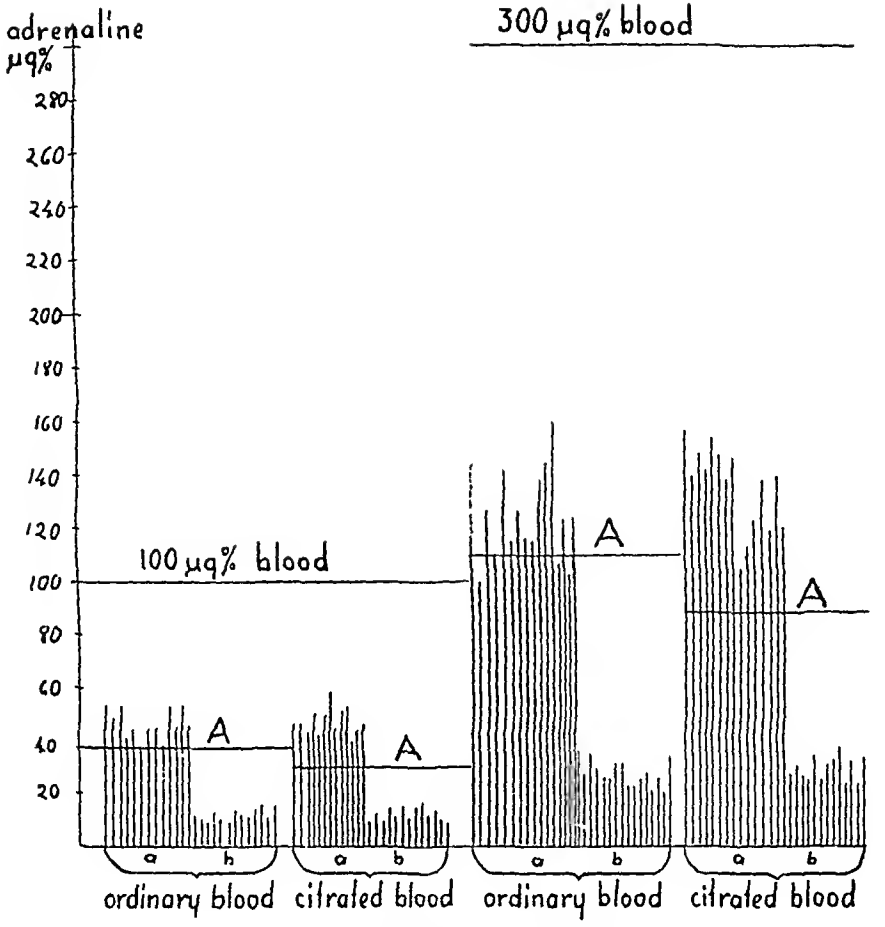

Fig. 28.
As above in fig. 27. 100 and 300 µg % adrenaline added to the blood.

aluminium hydroxide sediment (fig. 29). If in ordinary blood dilution the pH is above 7, adrenaline is adsorbed to the aluminium hydroxide in the same way as when the citrated blood dilution has a pH above 6.5, but remains in solution at a pH below these values. The adsorption zone of adrenaline is not strictly limited, since the equilibrium of pH in the recipient fluid varies somewhat in different tests.

The smallest adrenaline content which is demonstrable in the fluorescence reaction by dialysis methods is 5 µg % but in aqueous solutions, on the other hand, the fluorescence reaction of a 1 µg % adrenaline solution can be seen. This difference is due

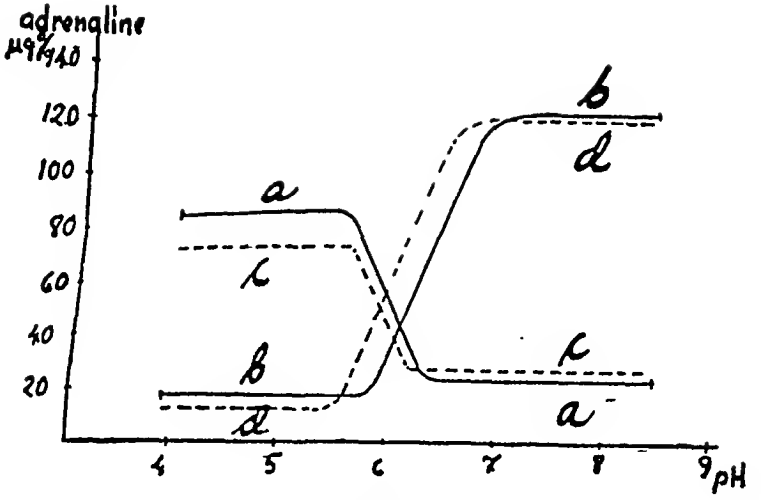

Fig. 29.

The amount of adrenaline in the aluminium hydroxide precipitate and in the filtrate after dialysis in different pH-ranges of ordinary and citrated blood solutions. 200 μg % adrenaline added to the blood.

a = filtrate of ordinary blood solution
b = precipitate „ „ „ „
c = filtrate „ citrated „ „
d = precipitate „ „ „ „

to the adrenaline becoming diluted in the dialysis process, in KALAJA and SAVOLAINEN's method (1941 a, c) 2.7 times and in JÖRGENSEN's method (1945, 1948) 3.4 times. Thus after dialysis of blood containing 2.7 μg % and 3.4 μg % adrenaline its content in the recipient fluid is 1 μg %. If it were a question of a pure aqueous solution the fluorescence reaction of an 1 μg % adrenaline solution would be easily discernible, but the estimation of fluorescence reactions of adrenaline after dialysis from blood in the recipient fluid is disturbed by several nonspecific fluorescent substances. In practice it is possible to determine in the recipient fluid the fluorescence reaction of 1.5—2 μg % adrenaline which corresponds to 4—5 μg % adrenaline in the blood to be investigated. Practically speaking it is the lower limit for the determination of adrenaline in the dialysis methods. On the other hand it is already easier to determine the fluorescence reaction of adrenaline in the recipient fluid, when 10 μg % adrenaline has been added to the blood solution.

When determining small adrenaline contents colorimetry errors must also be taken into consideration. The accuracy of the eye is poor when distinguishing weak fluorescence reactions as mentioned above.

Since nonspecific fluorescence substances in the dialysing fluid also disturb the estimation of small contents of adrenaline, endeavours must be made to eliminate these factors.

Although technically speaking all real sources of by-fluorescences are carefull cleaned, there remains in the recipient fluid a faint fluorescence of the same type as the fluorescence reaction of adrenaline, which on addition of alkali remains unchanged, does not disappear but slowly gains in intensity, being noticeable in samples to which alkali has been added a day or two later. This fluorescence originates from the blood. It is not found in samples dialysed under similar conditions, where the dialysis is carried out from distilled water instead of blood. Serum contains compounds, which give blue and yellow fluorescence (BUCHLOCH, 1938). By centrifuging blood corpuscles separately from plasma one is able to ascertain that the blue fluorescence originates chiefly from erythrocytes, and is derived from substances formed by the hemolysis of red blood corpuscles.

4. Author's adrenaline determination with the help of aluminium hydroxide adsorption.

A. *Development of the adsorption method.*

Since by the dialysis method the dilution of the adrenaline during dialysis prevents a determination of small quantities of adrenaline, it was deemed advisable to develop the dialysis method further by making use of the adsorption of adrenaline in the recipient fluid to aluminium hydroxide. On the basis of this adsorption ascertained by previous experiments endeavours have been made to develop a method by which adrenaline could be determined direct from the centrifuged aluminium hydroxide. When alkali is added the blue fluorescence disappears as the aluminium hydroxide dissolves and the fluorescence reaction of adrenaline becomes visible.

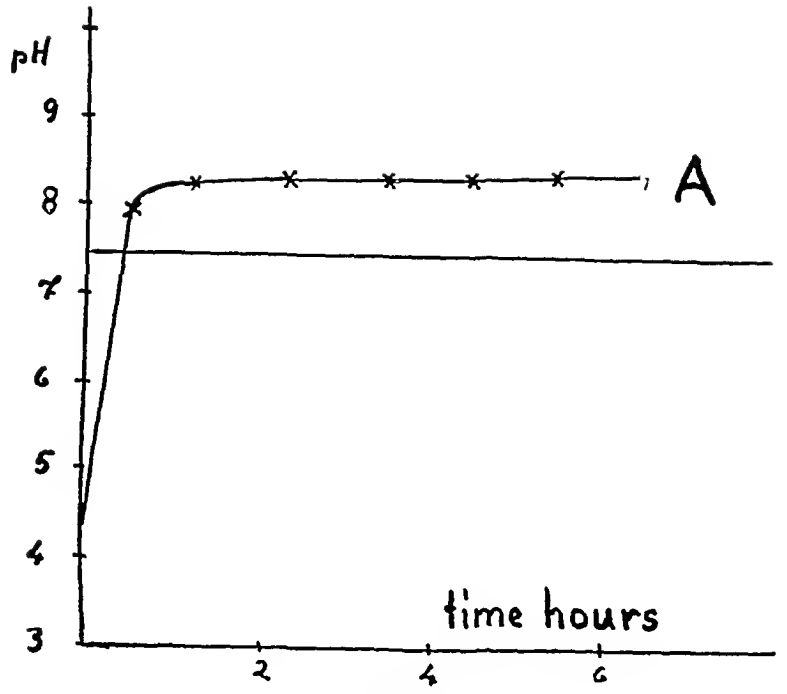

Fig. 30.
Rise of the pH in the recipient fluid in adsorption method during dialysis.

In the adsorption method the following factors must be taken into consideration: the amount of substances to be adsorbed must be made as large as possible and the adsorption as complete and as uniform as possible. It proved best to employ as blood solution 10 ml whole blood containing 0.5 ml saturated sodium citrate. From this blood adrenaline is dialysed into 10 ml distilled water, to which has been added 0.5 ml aluminium hydroxide suspension. If 10 ml whole blood is used, larger amounts of adrenaline are dialysed than in earlier dialysis methods. When carrying out the dialysis from the blood into equal amounts of distilled water, more adrenaline can be made to dialyse through the cellophane membrane, than in earlier dialysis methods.

The above arrangement also gives the pH which is optimal for adrenaline adsorption. At a pH 7.5 of whole citrated blood the pH of the recipient fluid has risen from 4.5 to 8.5 during completed dialysis. This happens already at an early stage of the dialysis, as illustrated by fig. 30, and it is higher after completed dialysis than the pH of the citrated blood. Citrated blood has a

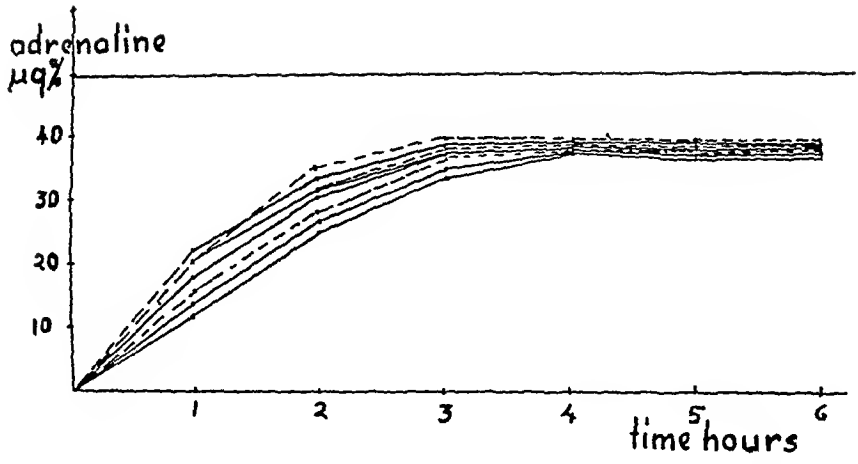

Fig. 31.
Dialysability of adrenaline from whole citrated blood against a corresponding volume of distilled water. Content of adrenaline in the recipient fluid during dialysis. 100 µg % adrenaline added to the blood.

tendency to raise pH of the recipient fluid higher than its own original pH. The pH rises also to the same level in the use of 1 ml aluminium hydroxide. Nor does an amount of 2 ml aluminium hydroxide yet prevent the rise of pH to the same level.

Adrenaline is dialysed from whole citrated blood almost as well as from diluted blood, since in this method the dialysis surface and the volume of recipient fluid are large. Fig. 31 illustrates the dialysability of adrenaline from whole citrated blood. As was the case in previous experiments with diluted blood, no complete equilibrium is found for adrenaline during dialysis also from citrated blood to distilled water. This is due to the adrenaline-retaining properties of proteins and to the *Donnan*-equilibrium.

If 0.5—2 ml aluminium hydroxide is added to the distilled water used as recipient fluid, adrenaline dialyses better from the blood than if mere water is used. The addition of aluminium hydroxide improves the effect of dialysis by about 1.5 times as shown in fig. 32. The adrenaline is adsorbed during the dialysis to the aluminium hydroxide which enables adrenaline to be continually dialysed from the blood.

The adsorption of adrenaline to the aluminium hydroxide is sensitive to all variations. The pH has to remain optimal. When

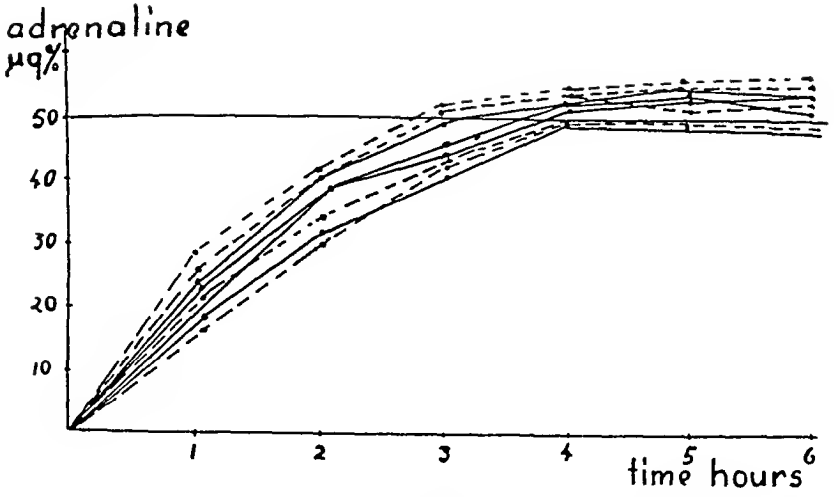

Fig. 32.
Dialysability of adrenaline from whole citrated blood against a corresponding volume of distilled water containing 1 ml aluminium hydroxide suspension. Content of adrenaline in the recipient fluid during dialysis 100 µg % adrenaline added to the blood.

adrenaline is dialysed from the citrated blood to distilled water containing aluminium hydroxide, the pH of this fluid is adjusted thanks to the ions dialysed from the citrated blood to the optimal level for the adsorption of adrenaline to the aluminium hydroxide; it has an average of 8.5 and can vary from about 8—9. If endeavours are made to reproduce the above-mentioned experimental conditions, *e.g.* by using phosphate buffers, the results are not as good as those obtained in experimental circumstances produced by the above arrangement.

In order to achieve good adsorption of adrenaline endeavours must be made to choose such an addition of aluminium hydroxide to the recipient fluid that the adsorption proceeds as satisfactorily as possible. Consequently the addition of aluminium hydroxide has to be sufficient to adsorb all adrenaline but not so large that the pH can not rise high enough. 0.5—1 ml has proved to be the most convenient addition of aluminium hydroxide (fig. 33). 0.25 ml aluminium hydroxide is already unable to adsorb all adrenaline from the dialysis fluid and 2 ml aluminium hydroxide is too large a dose as it can weaken the fluorescence reaction.

After completed dialysis the recipient fluid has to be centrifuged. As adsorption control 2 ml clear filtrate are placed in a

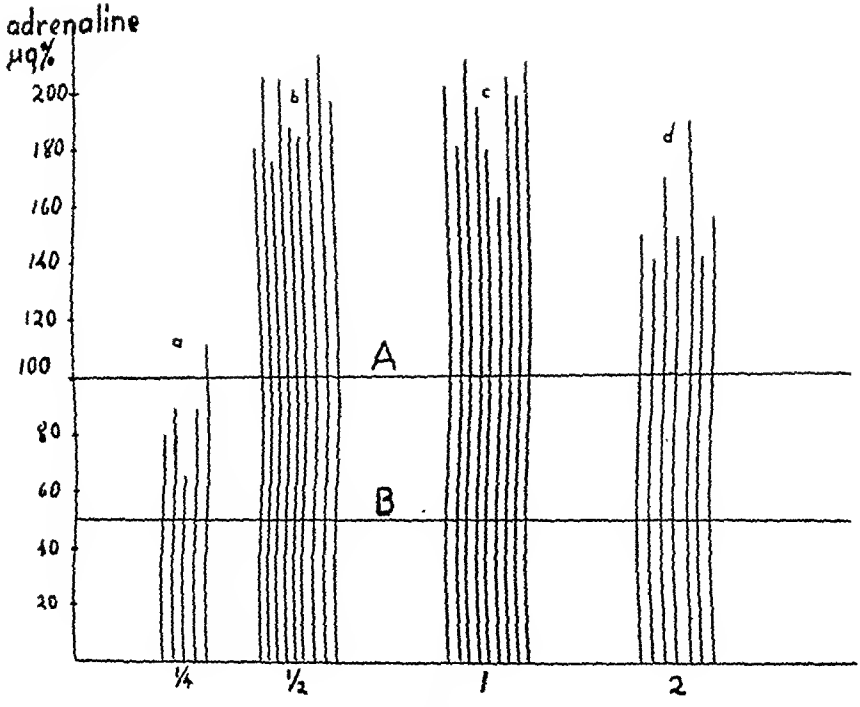

Fig. 33.

Content of adrenaline in the aluminium hydroxide precipitate in adsorption method after dialysis from whole citrated blood. 100 µg % adrenaline added to the blood.

a = 0.25 ml aluminium hydroxide in 10 ml distilled water as recipient fluid
b = 0.5 „ „ „ „ „ „ „ „ „ „ „
c = 1.0 „ „ „ „ „ „ „ „ „ „ „
d = 2.0 „ „ „ „ „ „ „ „ „ „ „
A = content of adrenaline in the blood before dialysis
B = expected content of adrenaline in the recipient fluid after dialysis.

test tube. The surplus clear filtrate is then carefully poured from the centrifuged tubes without removing the aluminium hydroxide precipitate from the bottom. The surplus on top of this precipitate is then removed with a small slip of blotting paper. The precipitate is then suspended in 1 ml distilled water and thereafter poured into a test tube for the determination of the fluorescence reaction of adrenaline.

On addition of alkali the fluorescence reaction of adrenaline develops more slowly in the aluminium hydroxide suspension than in the filtrate. An addition of 0.4 ml alkali is considered suitable to dissolve the aluminium hydroxide precipitate quickly and to cause the fluorescence reaction of adrenaline in this so-

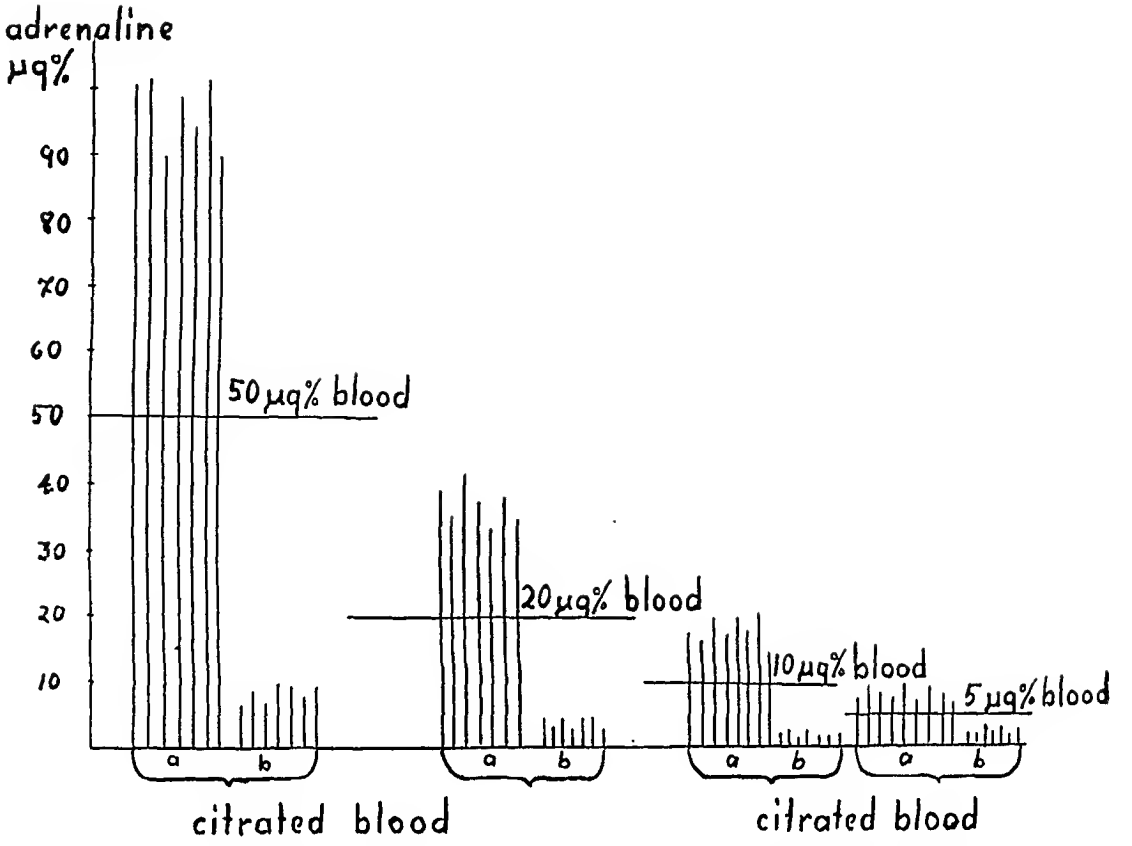

Fig. 34.
Content of adrenaline in the aluminium hydroxide precipitate (a) and in the filtrate (b) in adsorption method after dialysis from whole citrated blood. 5, 10, 20 and 50 μg % adrenaline added to the blood.

lution. The maximum reaction appears in 1—3 minutes depending on the content of adrenaline. Fig. 34 presents the results obtained from the filtrate as well as from the aluminium hydroxide precipitate after dialysis of blood samples containing 5, 10, 20, 50 μg % adrenaline. The results also show the adsorption effect in this method *i.e.* to what extent adrenaline has become adsorbed from the recipient fluid to the aluminium hydroxide.

With the help of the adsorption method much smaller adrenaline contents from the blood can be determined than by earlier dialysis methods. The fluorescence reaction can still be ascertained when dialysing adrenaline from the blood containing 0.5—1 μg % adrenaline. 5 and 10 μg % adrenaline can be very easily estimated, since the adrenaline is concentrated by adsorption to

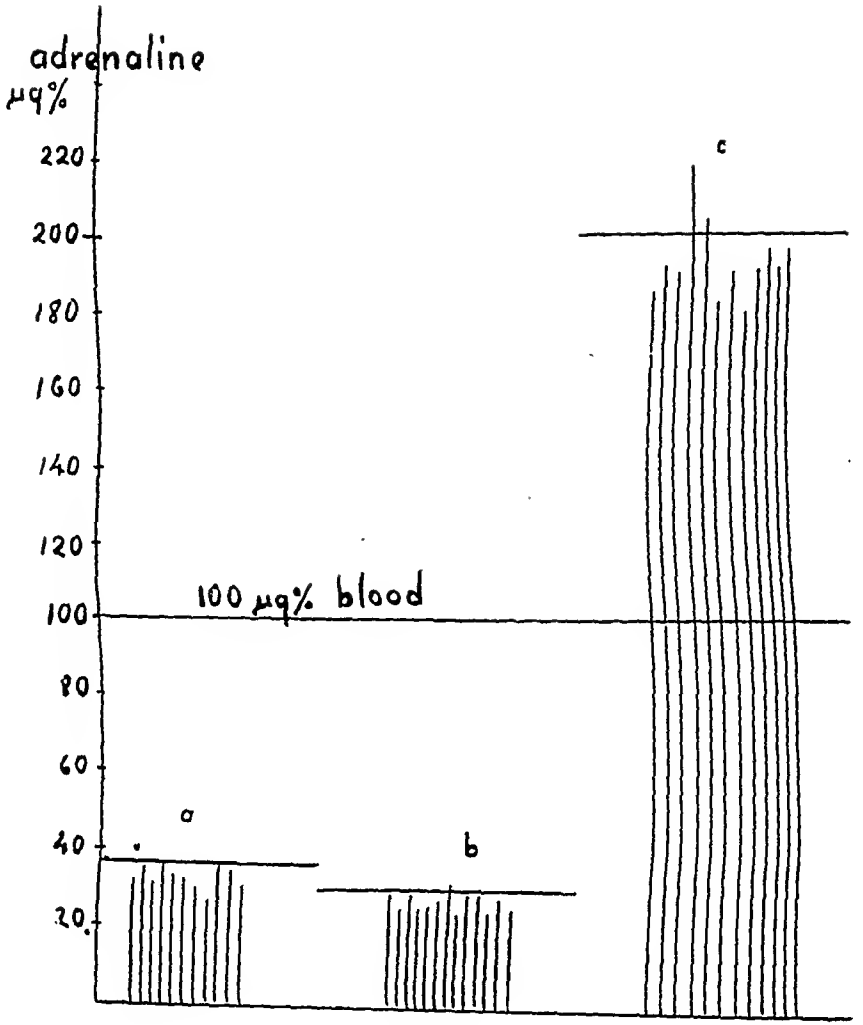

Fig. 35.
Results obtained with modified dialysis methods (a, b) in the filtrate and with the adsorption method (c) in the aluminium hydroxide precipitate. 100 µg % adrenaline added to the blood.

the aluminium hydroxide. In the suspended precipitate the adrenaline content is twice that of the citrated blood to be dialysed.

After dialysis of adrenaline from blood with 100 µg % adrenaline content first with the methods, in which pH is reduced so that adrenaline is not adsorbed to the aluminium hydroxide precipitate, and then with the adsorption method the results as shown in fig. 35 can be obtained. The same results are presented graphically

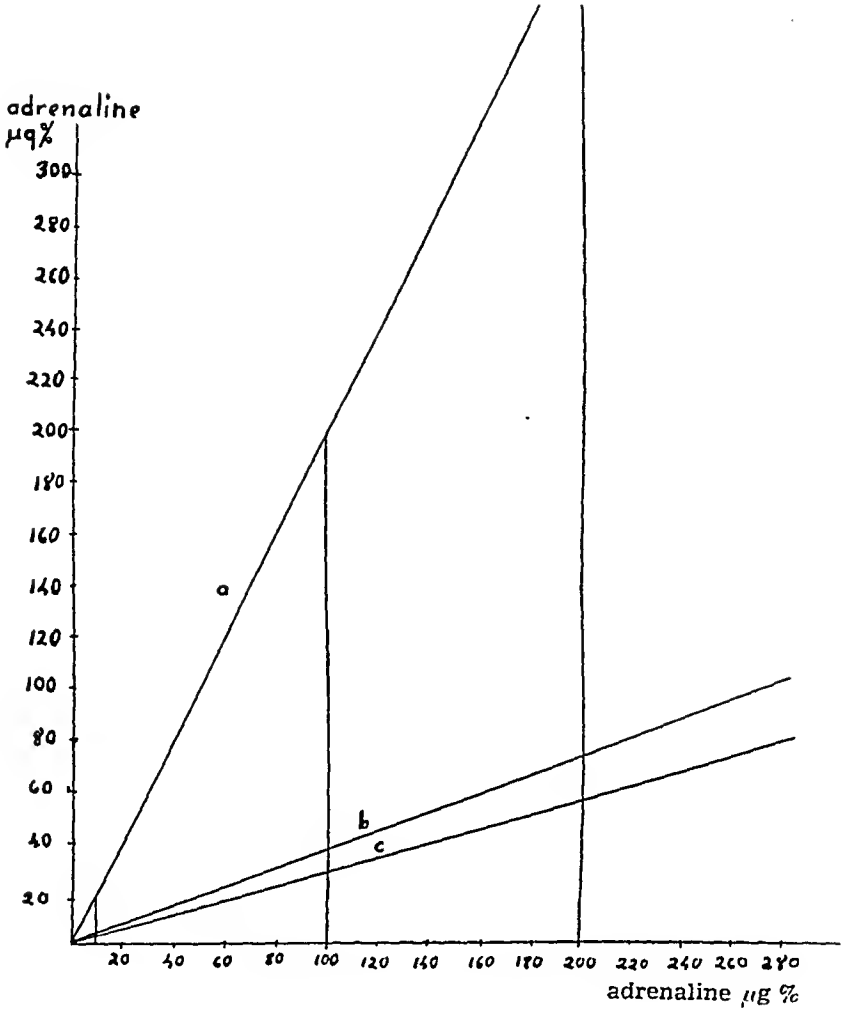

Fig. 36.

Graphic presentation of results (ordinate) obtained with modified dialysis methods in the filtrate (b, c) and with adsorption method in the precipitate (a). Abscissa = content of adrenaline added to the blood.

in fig. 36, which also gives the adrenaline content of the blood to be examined and of the sample after dialysis. It is possible to determine 5.4—6.8 times smaller adrenaline contents from the blood with the help of the adsorption method than with the modified dialysis methods.

The quantity of citrate to be added to the blood is also of importance for the adsorption of adrenaline to the aluminium

hydroxide, since the pH of the recipient fluid is dependent on the amount of citrate in the blood. The more citrate is used, the higher is the rise of pH in the recipient fluid after dialysis, and the less citrate is used, the less the pH of this fluid is able to rise above the initial level. An addition of 0.5 ml saturated sodium citrate in 10 ml whole blood has proved to be the most appropriate.

Aluminium hydroxide is one of the adsorbents most frequently used in chemical research. The adsorption characteristics of aluminium hydroxide can vary to some extent. They appear to be somewhat better in an aluminium hydroxide suspension which has been stored for some time than in a recently prepared solution. Apparently variations can take place in the size and solubility of the particles of aluminium hydroxide. The adsorbing capacity of a suspension of aluminium hydroxide which has been kept in a warm place diminishes. Solutions of aluminium hydroxide kept in a refrigerator seem to have a somewhat better capability to adsorb adrenaline than solutions which have been kept at room temperature. A fall in temperature promotes the adsorption. The dialysis of blood solutions and the centrifuging of the aluminium hydroxide precipitate had therefore best to be performed near 0°C, *i.e.* by placing the shaker and the centrifuge into a refrigerator.

B. *Technique of the adsorption method.*

Solutions:

1. Saturated sodium citrate solution which is non-fluorescent in UV-light.
2. Aluminium hydroxide suspension which is prepared according to SHAW (1938): 25 g potassium aluminium sulphate are dissolved in 200 ml water by heating and the solution is filtered and cooled to room temperature. 5 g sodium hydroxide dissolved in 20 ml distilled water are added in small doses to an alum solution stirring carefully. The solution in which the aluminium hydroxide is then precipitated must be filtered, the precipitate repeatedly washed with distilled water and lastly suspended in 100 ml distilled water. Aluminium hydroxide suspension may not fluoresce in UV-light after alkali has been added and the particles are dissolved.

2. 20 μg % eosin standard solution for the eosin test tube series.
4. 5 N sodium hydroxide solution which does not fluoresce in UV-light.
5. Distilled water which does not fluoresce in UV-light.

Equipment:

1. Dialysis dishes, the structure of which is explained above p. 39.
2. A cellophane membrane, about 17 mμ thick which must be cleaned in a weak acid and distilled water.
3. Rubber ring layers for tightening, which do not cause fluorescence.
4. Shaker (120 movements/min, amplitude 5 cm).
5. Thin-walled, non-fluorescent test tubes whose inner diameter is exactly the same (1 cm), for the determination of the fluorescence reaction of adrenaline in the solutions under examination and for the eosin test tube series.
6. Burette for alkali with a graduated scale, the volume of which is 2 ml.
7. Series of eosin test tubes, in which the eosin contents rise by intervals of 10 %. The contents are marked graphically on the tables p. 28. It must be prepared weekly.
8. Frame for eosin test tubes, movable on rails.
9. Ultra-violet lamp with a violet filter absorbing the visible rays of the lamp.

Procedure:

12 ml blood is withdrawn from the vein into a test tube on the bottom of which 0.5 ml saturated sodium citrate solution dried from water has been placed to prevent coagulation. The blood is well mixed with the citrate. Into one half of the dialysing chamber 10 ml of this sample is placed by means of a syringe with a long needle, and into the other half, which is fitted with two tubes, 10 ml distilled water and 0.5 ml aluminium hydroxide suspension, taking care that the needle does not tear the cellophane membrane. The side tubes are closed with clips. The samples are placed in the shaker in an erect position, in such a way that the plane of the cellophane membrane runs

parallel to the direction of movement. After shaking for 5 to 6 hours the adrenaline attains its equilibrium on both sides of the cellophane membrane, whereafter the suspension containing aluminium hydroxide and distilled water is poured out through one of the side tubes into the centrifuge tube, taking care that all the aluminium hydroxide is mixed and removed from the bottom of the dialysing dishes. The aluminium hydroxide suspension is centrifuged for five minutes. The centrifuge has ca. 3000—3500 rev/min. 2 ml of the clear filtrate is transferred by pipetting into a test tube for the determination of the fluorescence reaction of adrenaline, as a control to show that adrenaline has become adsorbed from the solution to the aluminium hydroxide precipitate. The surplus clear filtrate is poured from the centrifuge tube. For the adrenaline determination itself the aluminium hydroxide suspension on the bottom of it is carefull dried with a slip of blotting paper, and thereafter suspended into 1 ml distilled water. This suspension is poured into a thin-walled non-fluorescent test tube of exactly the same diameter.

The fluorescence reaction of adrenaline is determined by adding to both samples from a burette 0.4 ml 5 N sodium hydroxide and the fluorescence reaction is estimated at the height of its intensity with the help of the eosin test tube series. The maximum appears in the sample containing aluminium hydroxide in about one minute, and in the control filtrate in about half a minute. The appearance of the maximal intensity of this reaction is dependent on the adrenaline concentration of the samples to be investigated. The accuracy of determination is about 10—15 %.

C. *Experiments with other substances for adrenaline adsorption.*

WHITEHORN (1935), GIORDANO and ZEGLIO (1939) and KOBRO (1946 a) have made use of silicic acid for the adsorption of adrenaline.

The use of silicic acid adsorption in the above experimental arrangement in different pH zones did not yield such good results as when aluminium hydroxide adsorption was used. It must be taken into consideration that when determining adrenaline by means of the fluorescence reaction, the adrenaline

must be eluted from silicic acid with quite a strong acid, which may weaken or entirely prevent the fluorescence reaction. On the other hand, if adrenaline is adsorbed to aluminium hydroxide, it is not necessary to elute it, since aluminium hydroxide is dissolved on addition of alkali and thus does not interfere with the reading of the adrenaline fluorescence reaction.

Adrenaline and adrenochrome are absorbed to active carbon (GREEN and RICHTER, 1937) from which they can be extracted with methyl alcohol.

When using this method, the fluorescence reaction is not as strong as if adrenaline is estimated direct on addition of alkali from aluminium hydroxide suspension, yet it is better than after elution from silicic acid. Aluminium oxide also has a moderate adsorption of adrenaline at a pH of 7, but it is difficult to get adrenaline to elute from aluminium oxide. If fuller's earth is used as adsorbent, substances are dissolved into the elution fluid which are precipitated on addition of alkali, thus disturbing the determination of the adrenaline fluorescence reaction. The tests carried out above showed aluminium hydroxide to be the best adsorbent of adrenaline under these conditions. Moreover, aluminium hydroxide has no disturbing effect on the fluorescence reaction of adrenaline in the adsorption method.

D. *Modification of the adrenaline adsorption method.*

It is possible to determine a still smaller adrenaline content by the adsorption method by for instance increasing tenfold the amount of the citrated blood and recipient fluid containing aluminium hydroxide. On completed dialysis there is in the recipient fluid 10 times the quantity of adrenaline of the adsorption method. This adrenaline is adsorbed during the period of dialysis to the aluminium hydroxide, as soon as the pH of the recipient fluid rises to the level of optimal adsorption. After centrifuging the aluminium hydroxide precipitate is suspended with 10 ml distilled water. In the determination of the fluorescence reaction test tubes with a diameter of about 3 cm must then be used and 4 ml alkali will have to be added in conformity. When applying this method better results are achieved than in the use of smaller amounts

of blood; there is, however, no direct proportion to the amount of blood.

The method, if carried out in this way, is however inconvenient. Therefore it is more appropriate to carry out an elution of adrenaline from the aluminium hydroxide precipitate after centrifuging into 1 ml 0.2 N hydrochloric acid solution. The pH of the aluminium hydroxide precipitate is then reduced to a level suitable for the elution of adrenaline. By repeating the elution with the same amount of hydrochloric acid solution also the remaining part of the adrenaline from the aluminium hydroxide precipitate can be eluted into it. By proceeding thus it is possible to increase the sensitivity of the adsorption method 2—3 times. This method is suitable if large amounts of blood are available and if such a small adrenaline content has to be determined, that it is not demonstrable by applying the ordinary method of aluminium hydroxide adsorption. Nor does in the elution method the aluminium hydroxide prevent the visibility of the initial fluorescence in the dialysing fluid before addition of alkali, as is the case in the determination of adrenaline direct from aluminium hydroxide suspension, whose blue fluorescence completely conceals the initial fluorescence before the addition of alkali.

In the original method of aluminium hydroxide adsorption it is also possible to elute adrenaline from the centrifuged aluminium hydroxide precipitate with 1 ml 0.1 N hydrochloric acid solution and in that way prevent the disturbing influence of the aluminium hydroxide precipitate in the reading of the initial fluorescence of the dialysing fluid.

E. *Determination of adrenaline in central and peripheral blood.*

As illustrated by the results of both biological and chemical methods in the historical survey, the normal adrenaline content of peripheral blood is uncommonly small. Several workers are of the opinion that there is practically no adrenaline in peripheral venous blood. Many a result obtained by chemical methods may be due to *nonspecificity.*

It should be possible to determine by adsorption methods those adrenaline contents which had been found in peripheral venous blood by earlier fluorescence methods and some chemical methods. By the method just described, 0.5—1 μg % adrenaline added to blood can be determined, whereas the above mentioned results in peripheral venous blood vary from a few μg % to some hundred μg %. Yet it was not possible in the researches carried out to demonstrate by this adsorption method any values of adrenaline in peripheral venous blood, either in man or in test animals. Therefore, the adrenaline content of peripheral venous blood must be under 0.5 μg %. Nor was it possible to demonstrate adrenaline by the adsorption method in normal arterial blood.

Since the results obtained from peripheral blood proved to be negative, samples have also been taken from the upper course of the inferior vena cava above the suprarenal vein, where the highest adrenaline content is assumed theoretically. When samples are taken there, the adrenaline has not had time to reach the capillary area or to be adsorbed by the tissues; instead it flows from the suprarenal vein in a thin slow stream into the wide channel of the inferior vena cava and is gradually mixed with the blood in the latter. When taking samples under ether or evipan anæsthesia by means of a cannula, in such a way that the point was on a level with the lower part of the sternum, no adrenaline could be found under normal conditions in rabbits, dogs or even calves. If on the other hand samples are taken after laparotomy, 1—5 μg % adrenaline can be found in the inferior vena cava in some samples, evidently due to increased adrenaline secretion in consequence of surgical manipulation and local handling, but in some samples no adrenaline was found. Evidently the head of the cannula may sometimes encounter a place in the blood stream where there is adrenaline, and sometimes one where there is none at all. It is therefore evident that the adrenaline content of the inferior vena cava does not under normal conditions exceed the sensitivity limit of the adsorption method, although one may find a little adrenaline in some samples of blood from inferior vena cava under certain conditions of increased adrenaline secretion.

II.

THE SECRETION OF ADRENALINE FROM THE SUPRARENAL GLANDS.

Earlier Investigations.

Researches on the secretion of adrenaline from the suprarenal glands yield a clearer and more uniform picture than researches that were carried out for the determination of adrenaline in peripheral blood. This is due to the circumstance that it is much easier to determine adrenaline from the suprarenal vein where the adrenaline content is greater than in the peripheral blood.

The first to have estimated the adrenaline content of the suprarenal vein by biological methods was EHRMANN (1905). He obtained the value of 100 μg % by the enucleated frog eye method. TRENDELENBURG (1911) seems to have been the first to determine the adrenaline secretion from the suprarenal vein. He found by the frog leg method the secretion value of 1.6 μg/kg/min in cats (adrenaline secretion is usually calculated in μg per kilogram of body weight per minute). This value was strikingly high, since no vessel-constricting substances in blood coagulation had been taken into consideration by this method. For the collection of suprarenal venous blood STEWART and ROGOFF (1916) using the cava pocket, obtained an adrenaline secretion of 0.23 μg/kg/min and KODAMA (1923) by the same method 0.55—1—0.35 μg/kg/min in cats, dogs and rabbits.

In these determinations no consideration was paid to changes due to anaesthesia, surgical manipulation or cava pocket and therefore the values for the resting secretion were comparatively high. When examining the conditions of resting secretion endeavours must be made to preserve as much as possible the physiological test conditions. Instead of anæsthesia

6. Author.

1.	2.	3.	4	5.	6
		Stimulation of splanchnic nerve		1—2 rabbit 8—20 cat 50—75 dog	total secretion TRENDELENBURG, 1925
		—„—			
		—„—			
	0.2-0.4	Severance of splanchnic nerve		0,007—0,004	STEWART and ROGOFF, 1917
		—„—		0.05—0.07	LEWIS and PRIETO, 1939
10—100	0.7	Stimulation of sensory nerves		3.2—3.7	CANNON and RAPPORT, 1921
60—120	0.6	—„—	60—640—1400	1.3—4.7	KODAMA, 1923
16	0.2	—„—		0.8	STEWART and ROGOFF, 1924
30—60	0.3-0.6	—„—	100—200—400	1—3	KODAMA, 1924 a
	0.7	—„—		1.1—3.6	SUGAWARA, WATANABE and SAITO, 1926
5—15	0.02	—„—	30—100—170	0.5—6.4	SATAKE, WATANABE and SUGAWARA, 1927
10—100	0.6	Asphyxia	200—400—600	2—4	KODAMA, 1924 c
10—40		—„—	200—330—600	2.2	HOUSSAY and MOLINELLI, 1926 a
5—10	0.04	—„—	40—260—480	0.4—1.4	SATO, INABA and TAKAHASHI, 1932
		Nicotine		2—2.5	CANNON, AUB and BINGER, 1911
14	0.3	—„—	130—400	2	STEWART and ROGOFF, 1919 b
10—40		—„—		1—5	HOUSSAY and MOLINELLI, 1926 b
5—10	0.02	—„—	15—45	0.1—0.3	WATANABE, 1935 a
5—10	0.02	—„—	40—1280—2560	0.7—10	WADA, HIRANO and TIBA, 1938
12— 20	0.2	Strychnine	100—200	0.7—1.5	STEWART and ROGOFF, 1919 a

		—„—		1—1.5	TRENDELENBURG, 1923 a
		—„—		0.2	SHIMIDZU ,1924
15—30	0.03	—„—	80—475—900	0.8	WATANABE, 1927 a
10—15	0.02	—„—	30—60—70	0.2—0.06	AOMURA and YEN, 1929
20—35	0.03	Caffeine	60—600—1625	1—5	WATANABE, 1928 c
20—35	0.03	—„—	30—100—280	0.06—1.2	SATO and AOMURA, 1929
10—20	0.03	Morphine	40—60—110	0.03—1.7	STEWART and ROGOFF, 1922 a
5—10	0.02	—„—	40—70—160	0.07—0.5	SATO and OHMI, 1933
5—10	0.02	—„—	25—70—110	0.1—0.24	SATO, DEGTI and SATOW, 1935
5—10	0.02	—„—	30—60—160		WADA, TANAKA, HIRANO and TANEITI, 1938
		Insulin		0.5	ABE, 1924
5—15	0.03	—„—	70—120—190	0.1—0.7	YEN, AOMURA and INABA, 1933
5—10	0.02	—„—	80—240	0.2—0.4	SATO, OHMI and KANOWOKA, 1933
		Diabetic puncture		0.2—0.5	TRENDELENBURG, 1923 b
		—„—		0.2—0.7	SHIMIDZU, 1924
5—10	0.02	—„—	25—200	0.1—0.5	WADA, 1931
2—50		Ether		0.2—0.8	McCLURE and HOSKINS, 1912
40	0.4	—„—	70—200	0.5—1	KODAMA, 1924 b
50—200	0.8	Chloroform	40—80	0.34	KODAMA, 1924 d
		Operation		0.2—0.5	TRENDELENBURG, 1923 a
		—„—		0.23—0.27	STEWART and ROGOFF, 1923
5—10	0.02	Hemorrhages	60—150—450	0.3—0.4	SAITO, 1928 a
10	0.04	—„—	85—115	0.2	SAITO, KAMEI and TACHI, 1928
5—20	0.03	Peptonic shock	60—1000—2500	0.06—2.3	WATANABE, 1927 b
20—50	0.2-0.4	Anaphylactic shock		0.2—0.8	COHEN, RUDOLPH, WASSERMANN and ROGOFF, 1933
5—10	0.02	—„—	160—1920—4800	0.6—1	SATO, OHGURI and WADA, 1935
5—10	0.02	Histamine	160—320—960	0.3—0.7	WADA, FUZII, SIBUTA, SAKURAI and LI, 1940
5—10	0.01	Acetylcholine	30—50	0.2—0.7	INABA, 1935 a
20—60	0.3-0.6	—„—	110—640—1280	1—4	TANEITI, 1940
15	0.15	Physostigmine	200	0.7—1.4	STEWART and ROGOFF, 1921

1.	2.	3.	4	5.	6
5	0.01	Physostigmine	25—40	0.04—0.07	WATANABE, 1935 c
5	0.02	Pilocarpine	10—25	0.1—0.15	WATANABE, 1935 b
10—60	0.4	—„—	100—200—600	1—2	HIRANO, 1939
20—45	0.2	Tetrahydronaphtylamine	15—60—600	1—3	SUGAWARA, 1927
15	0.03	—„—	15—35	0.03—0.08	SATO and SUGAWARA, 1930
		Picrotoxin		0.9	SHIMIDZU, 1924
20—70	0.2-0.9	Diphteria toxin	60—130—225	0.09—2	WATANABE, 1928 b
30—35	0.2-0.9	Tetanus toxin	50—110—170	1—1.6	WATANABE, 1928 a
10—30	0.03	Guanidine	90—175	0.5	SUGAWARA and TADA, 1927
5—10	0.02	Digitalis	30—100	0.3—1	HATANO and SAIZYO, 1936 a
7—15	0.02	Urethane	15—35	0.05—0.1	SATO and AOMURA, 1930
40—140	0.7-2.0	—„—	40—120	0.1—0.3	KODAMA, 1930
10—50	0.02-0.1	Ephedrine	200—500	0.4	TAKAHASHI, INABA and WADA, 1935
		Santonin sodium		0.5	SHIMIDZU, 1924
		Camphor		0.3	SHIMIDZU, 1924
	0.03	—„—		0.05	OIKAWA and INABA, 1930
5—10	0.02-0.04	Rhodein	100—300	0.1—0.4	HATANO and SAIZYO, 1936 b
5—10	0.02	Sodium nitrite	30—60	0.1—0.2	SATAKE, SATO and ABE, 1937
120—300	1—2	Chloral	70—150	0.5—1	KODAMA, 1924 e
5—10	0.03	—„—	10—20	0.03	SAITO and OHMI, 1933
5—10	0.03	Cold		0.03	SAITO, 1928 b
5—10	0.03	—„—	60—120—200	0.3—0.4	WADA, SEO and ABE, 1935 a
5	0.02	Muscle work	20—45	0.03—0.1	WADA, SEO and ABE, 1935 b
10—60	0.3	Stimulation of carotid sinus nerve	10—20	0.1	AOMURA, 1930
10	0.01	Atropine	15—30	0.05—0.1	SATO, HATANO and MUTO, 1938
5—10	0.02	Avertin	15—20	0.03	SATO, SATOW and DEGTI, 1934
5	0.01	Veronal	10—30	0.05	SATO and DEGTI, 1935
5	0.01	Lobeline	15—20	0.05	INABA, 1935 b
		Tetrodotoxin			SATAKE, 1931
10—100					EHRMANN, 1906
20—100					O'CONNOR, 1911 a, b, c

KODAMA (1924 b) reverted to severance of the posterior roots of the spinal nerves in the abdominal region. SATAKE, SUGAWARA and WATANABE (1927) took their samples direct from the suprarenal vein and obtained an adrenaline secretion of 0.02 μg/kg/min. Approximately similar values were also obtained by other Japanese workers (SATAKE, WATANABE and SUGAWARA, 1927; SUGAWARA, SAITO and NEMOTO, 1927; SUGAWARA and TADA, 1927; WATANABE, 1927 a, b; SAITO, 1928 a, b; SAITO, KAMEI and TACHI, 1928; WATANABE, 1928 a, b, c; AOMURA, 1929; AOMURA and YEN, 1929; SATO and AOMURA, 1929, 1930; AOMURA, YEN and OIKAWA, 1930; OIKAWA and INABA, 1930; SATO and SUGAWARA, 1930; SATAKE, 1931; YEN, KAIWA and WADA, 1931; WADA, 1931; SATO, INABA and TAKAHASHI, 1932; SAITO and OHMI, 1933; SATO and OHMI, 1933; SATO, OHMI and KANOWOKA, 1933; YEN, AOMURA and INABA, 1933; SATO, SATOW and DEGTI, 1934; INABA, 1935 a, b; SATO and DEGTI, 1935; SATO, DEGTI and SATOW, 1935; SATO, OHGURI and WADA, 1935; TAKAHASHI, INABA and WADA, 1935; WADA and KANOWOKA, 1935; WADA, SEO and ABE, 1935 a, b; WATANABE 1935 a, b, c; HATANO and SAIZYO, 1936 a, b; SATAKE, SATO and ABE, 1937; SATO, HATANO and MUTO, 1938; WADA, TANAKA, HIRANO and TANEITI, 1938; WADA, FUZII, SIBUTA, SAKURAI and LI, 1940), according to which the adrenaline secretion at rest only varies within the limits of 0.02—0.05 μg/kg/min. BRENNING and AHLBORG (1939) also found an average resting secretion of adrenaline of 0.04 μg/kg/min.

Adrenaline is secreted in considerably greater quantities under the influence of several secretion stimuli. Table 2 illustrates the secretion values in normal and pathological states and the adrenaline contents in corresponding samples from the suprarenal vein.

Theoretical calculation of the adrenaline content in peripheral and central blood.

According to table 2, the strongest secretion stimuli are electrical stimulation of the splanchnic or sensory nerves, asphyxia, peptonic and anaphylactic shock, nicotine, strychnine, caffeine acetylcholine, pilocarpine, physostigmine, morphine, diphtheria toxin and tetanus toxin, after which the secretion of adrenaline can increase by some μg/kg/min. During an increased adrenaline secretion there are from some tens to some hundreds, sometimes even some thousands μg % adrenaline in the suprarenal vein. An average of 2—20 μg % adrenaline can thus be found in the above non-physiological states in individual samples from the inferior vena cava if the minute volume of the blood is about 100

ml per kg of body weight (TRENDELENBURG, 1923 a). It must, however, be taken into consideration that the high secretion values were found only in individual samples, and often under circumstances which do not correspond to physiological conditions. The electrical stimulation of the splanchnic, severed sciatic, median or brachial nerves is one of powerful non-physiological stimuli of adrenaline secretion. In asphyxia tests high secretion values were obtained only when the animal had been kept deeply asphyxiated for several minutes. The doses of drugs administered also surpassed many times the maximal.

Modern researches have shown that after injections of insulin, histamine, picrotoxin, santonin sodium, camphor, quanidine, sodium nitrite, ephedrine, rhodein and after CLAUDE BERNARD's diabetic puncture, ether anæsthesia, operation and cold stimulation 0.2—0.8 μg % on an average about 0.5 μg % adrenaline kg/min is secreted. The corresponding adrenaline content of the inferior vena cava is then 0.4—1.6 μg % on an average 1 μg %. This content diminishes still more in the area of the pulmonary and systemic circulation of the blood.

After lobeline, tetrodotoxine, veronal and atropine the adrenaline secretion but slightly surpasses the normal and is approximately the same after avertin and chloral as in the resting secretion.

According to the latest studies, the resting secretion of adrenaline in test animals varies only between 0.05—0.12 μg/kg/min. The adrenaline content in the corresponding samples from the suprarenal vein is on an average 5—10 μg %. When the blood from the thin suprarenal vein flows into the inferior vena cava, it becomes strongly diluted. In the light of these investigations, the content of adrenaline in inferior vena cava is low. If the minute volume of the blood is 100 ml, the corresponding adrenaline content of the inferior vena cava blood is then about 0.1—0.2 μg %, provided the adrenaline had distributed itself evenly in it. When flowing through the pulmonary and systemic circulation, adrenaline is dialysed in the capillaries into the tissues. Thus there will be less adrenaline in the peripheral venous blood than in the upper course of the inferior vena cava.

The adrenaline content in the peripheral blood circulation has thus to be very small, if it is taken into consideration that during the time of strongly increased adrenaline secretion there is in the inferior vena cava only the above average of 1 μg % adrenaline.

It is quite possible to demonstrate adrenaline in the suprarenal vein during resting and increased secretion and also in the upper part of inferior vena cava after increased secretion with the help of the adsorption method. The biological method most commonly used for this purpose was the rabbit intestine method, which was proved to be very specific and this has been employed extensively of late in the determination of adrenaline in the suprarenal vein. The adrenaline does not always rise to the same level but varies considerably even in successive samples taken from the same animal, and in different tests (table 2).

Author's Researches.

1. Determination of adrenaline in the upper part of the inferior vena cava.

Although the results of adrenaline determinations in peripheral blood are very variable, it was easiest to determine it in the suprarenal vein, and during the time of heightened secretion also in the inferior vena cava. Under stimulation the adrenaline secretion can rise considerably above the level of resting secretion, and it is then possible to determine adrenaline in the inferior vena cava as well. 2—8 μg % adrenaline could be determined in vena cava blood samples of rabbits and dogs taken after insulin and morphine injections from the upper part of the inferior vena cava, whereas there was no adrenaline at all in some parallel samples. The variations of adrenaline contents in different samples are probably due to the circumstance that the blood running from a thin vein into a large main vein has not yet reached its equilibrium, and the point of the needle can sometimes gather more adrenaline and sometimes none. As is evident from the above survey, the vena cava may contain an average of

1—2 μg % adrenaline after insulin or morphine injections. The results obtained approximately support these secretion values. The tests were carried out under ether anæsthesia.

2. Determination of adrenaline in the suprarenal vein.

In the dog, when taking samples by means of a thin syringe needle direct from the left lumbar vein, into which the suprarenal vein flows, the author was able to demonstrate in individual samples 1—10 μg % adrenaline under ether anæsthesia without any other stimuli to secretion. These tests prove that exceedingly small amounts of adrenaline are secreted into the blood stream by the suprarenal gland in the area of the suprarenal vein.

The above tests make it evident that the fluorescence method can be as well adapted to studies of adrenaline contents in the suprarenal vein as the biological methods used earlier. The experiments are somewhat impaired by the need of great quantities of blood in the dialysis methods, and the taking of blood samples is time-consuming, which makes it difficult to follow up the rapid variations in case of stimulations. The dog is a suitable test animal. Taking samples from rabbits already presents difficulties.

Since heart sounding is used as an aid in modern heart examinations, it would be conceivable to apply the same technique when taking samples from the inferior vena cava as well, and it would be possible to throw some light on the significance of adrenaline secretion in man both under normal and pathological circumstances, by using suitable stimuli of the adrenaline secretion, when it would also be possible to demonstrate small rises in the adrenaline contents of the inferior vena cava.

III

DISAPPEARANCE OF ADRENALINE FROM THE ORGANISM IN VIVO.

Earlier Investigations.

Subsequent to an adrenaline injection into the portal vein several workers have found that the effect of adrenaline on the blood pressure is considerably weaker than if it is injected into a vein leading direct to the heart, and they consider the liver to be the principal eliminator of adrenaline from the organism (LANGLOIS, 1897, BATTELLI, 1902 b, FALTA and PRIESTLEY, 1911, TRENDELENBURG, 1916, PAK, 1926, MARKOWITZ and MANN, 1929, GOLLWITZER—MEIER, 1930, SUGANUMA, 1934 c, SUNDBERG, 1928, HAYNAL, 1928, OGAWA, 1912, TANI, 1931, BATRAK, 1939, PHILPOT and CANTONI, 1941). In order to achieve a rise of blood pressure when making the injection into the portal vein, a 5 to 20 times larger amount of adrenaline is needed then if it is injected into the femoral or ear vein (GOLLWITZER—MEIER, 1930, OGAWA, 1925, TANI, 1931, HASEGAWA, 1933, PHILPOT and CANTONI, 1941.) Some investigators have found as well a diminished effect of adrenaline on the area of the peripheral muscle circulation. The blood pressure is lower after an injection of adrenaline into the femoral artery than into the femoral vein (CARNOT and JOSSERAND, 1902, LIVON, 1904 a, b, ELLIOT, 1905, MÖLLER, 1906, FALTA and PRIESTLEY, 1911, HESS, 1921, SUNDBERG, 1928, MARKOWITZ and MANN, 1929, GUTMAN, 1936, BATRAK, 1937, 1939.) The effect of adrenaline diminishes also in the area of pulmonary circulation (CARNOT and JOSSERAND, 1903, HÜLSE, 1922 a). This is not supported by all (ELLIOT, 1905, EMBDEN and FÜRTH, 1904, TRENDELENBURG, 1916, BATRAK, 1939, FALTA and PRIESTLEY, 1911.) Nor does the effect of adrenaline weaken in the area of the carotid blood circulation (FALTA and

PRIESTLEY, 1911). According to MARKOWITZ and MANN (1929) adrenaline is destroyed in the whole organism. In all these experiments weakening of adrenaline effect is partly due to the constriction of local capillaries in individual organs owing to which there is a slower flow of this hormone into the circulation (SUNDBERG, 1928).

If the liver is shut off from the circulation in such a way that the blood of the portal vein is directed straight into the vena cava (ATHANASIU and LANGLOIS, 1897, SUNDBERG, 1928) or hepatectomy carried out (MARKOWITZ and MANN, 1929, SUGANUMA, 1934 b), a rise in blood pressure due to the adrenaline injection is brought about, which has a longer persistence than in normal experimental animals. Also gastrectomy, splenectomy and eviscerectomy double the time of duration of the effect of adrenaline on the blood circulation (SUGANUMA, 1934 b), but a ligature of the renal blood vessels (JACKSON, 1909), and an excision of the kidney (SUGANUMA, 1934 c) do not have any influence on the adrenaline effect. An occlusion of the femoral and lumbar artery prolong the adrenaline effect much less than an occlusion of the portal vein (SUGANUMA, 1934 a, b). According to BACQ (1937 a), adrenaline is not destroyed at any greater rate by the liver and the abdominal viscera than by other tissues of the body. WEINSTEIN and MANNING (1937) claimed that adrenaline was dialysed into the tissues in the whole area of capillary circulation, and not only in the area of liver, spleen and kidney circulation, since the shutting off of these organs does not produce any particular alteration in the effect of adrenaline on the blood pressure.

After an injection of Indian ink, adrenaline has a stronger effect on the blood pressure than normally in test animals (UEMORI, 1930 a, 1932, 1933). This difference becomes more evident after an injection of adrenaline into the mesenteric than into the ear vein. The carbon particles of Indian ink, by adsorption to the reticuloendothelial system, prevent the adsorption of adrenaline into this tissue. Lithium carmine and colloidal silver have the same effect (UEMORI, 1930 b). The same rise is also observed after injections of methylene blue (PHILPOT and CANTONI, 1941), aromatic and aliphatic di- and monoamidine derivatives, di-guanidine and guanidine, as well as ephedrine, cocaine and thyramine (DAWES, 1946). These substances inhibit the amino-oxydase which destroys adrenaline in the organism (PHILPOT and CANTONI, 1941, DAWES, 1946).

In isolated liver (LANGLOIS, 1897, ELLIOT, 1905, BATTELLI, 1902 b, EMBDEN and FÜRTH, 1904, PAK, 1926, MACHII, 1932) through which a Ringer-solution is perfused, the adrenaline content of the perfusion fluid decreases. Adrenaline also disappears when passing through the frog-leg preparation (LIVON, 1904 a, b, LAEWEN, 1904, TRENDELENBURG, 1910). PAK (1926) and SUNDBERG (1928) were unable to support this observation. EMBDEN and FÜRTH (1904) maintain that only a little adrenaline disappears from the isolated lung, but none at all according to ELLIOT (1905) and HARADA (1925). In perfusion of Ringer's solution through

different organs, the disappearance is quickest in the liver, then in the spleen, lungs and intestine, and slowest in the bones (MACHII, 1932). In perfusion through the portal vein the loss of adrenaline is 10 times as much as in perfusion through the hepatic artery (MACHII, 1932).

Several investigators have observed the rapid disappearance of adrenaline from the systemic circulation after an adrenaline injection. Several of these experiments were made using biological methods (OLIVER and SCHÄFER, 1894, EHRMANN, 1905, ELLIOT, 1905, VOS and KOCHMANN, 1905, FALTA and FLEMING, 1911, FALTA and PRIESTLEY, 1911, O'CONNOR, 1911 c, BORBERG, 1912, TRENDELENBURG, 1916, HÜLSE, 1922 a, LICHTWITZ, 1922, SCHLOSSMANN, 1927, FROMHERZ, 1927, SATO, 1930, ROGOFF and MARCUS, 1938, WERLE, 1938, COHEN, 1945).

When injecting 1 mg adrenaline into the circulation of the cat, 80 % of it disappears within three minutes by measuring with the blood pressure method (ELLIOT, 1905). When infusing adrenaline into the systemic circulation, the adrenaline content of arterial blood is only 1/4—1/16 of the expected amount, whereas no adrenaline could be found in the venous blood which had passed through the capillaries (TRENDELENBURG, 1910, O'CONNOR, 1911 c, BORBERG, 1912). Adrenaline rapidly disappears also from the arterial blood immediately after the infusion. Disappearance proceeds in the capillary area so rapidly, that none can be demonstrated in venous blood during infusion, or at least considerably smaller amounts than in arterial blood (HESS, 1922, HÜLSE, 1922 a, c. SCHLOSSMANN, 1927, SATO, 1930).

Subsequent to an intravenous administration of 300 μg adrenaline/kg body weight, corresponding to an adrenaline content of about 300 μg % providing it distributes itself evenly in the whole circulation, no adrenaline could, however, be determined after 5—13 minutes in the dog's blood by the rabbit intestine method (SATO, 1930).

When injecting adrenaline about 3 μg/kg/min, i.e. 15 times the rapidity of the suprarenal adrenaline secretion, as supposed by STEWART and ROGOFF (1923) or 150 times the amount which is now considered to be secreted at rest, ROGOFF and MARCUS (1938) found in arterial blood 0.5 μg % adrenaline. The veins of the systemic and pulmonary circulation have only about half of the arterial adrenaline content. Immediately on completion of the injection the adrenaline content of arterial blood is 0.12 μg % and after one circulation the adrenaline has completely disappeared from the blood. During one circulation time the disappearance or adrenaline is nearly 100 %.

When injecting adrenaline 7—15 μg/kg/min or 25—50 times the suprarenal resting secretion as assumed by STEWART and ROGOFF (1923) or 250—500 times the amount which is now considered to be secreted at rest, the adrenaline content immediately on completion of the injection is only 1/4—1/2 of the content during infusion. In spite of continued infusion, the

adrenaline in the blood rises only to a certain threshold level. ROGOFF and MARCUS (1938) maintains that it is not arithmetically possible for such amounts of adrenaline to be present physiologically in the blood circulation, as have been demonstrated by several workers with the help of their methods. Such adrenaline contents continually present in the blood cause the death of the test animals within some days or hours (DRAGSTEDT, v. PROHASKA and HARMS, 1937 a, b, ROGOFF and MARCUS, 1938).

According to COHEN (1945) the disappearance of adrenaline from the dog's blood subsequent to intravenous injection follows the hyperbolic curve and 90 % of adrenaline disappears in the course of the first eight minutes.

The disappearance phenomenon of adrenaline has been studied by chemical methods by BLOOR and BULLEN (1941 a). Before them WHITEHORN (1935) pointed out that no adrenaline could be found in peripheral venous blood, while it was found after stimulation of suprarenal secretion in the blood of the vena cava and of the arteries. By the arsenomolydbic acid method BLOOR and BULLEN (1941 a) could recover one minute after an intravenous adrenaline injection on an average 25 % adrenaline, within five minutes 21 % and and within 20 minutes 14 %, but after 20 minutes there was no adrenaline at all in the samples.

Some investigators have also studied the possible secretion of adrenaline into the urine, by endeavouring to make a chemical determination of adrenaline-like substances in urine. When giving adrenaline per os a substance was found in urine, yielding a positive ferric chloride reaction, and increasing the blood pressure (FALTA and IVCOVIC, 1909). According to WEINSTEIN and MANNING (1937), adrenaline which has been dialysed into the tissues through the capillaries and become oxidised there to protocatechuic acid, is eliminated in combination with phenol in the urine. RICHTER (1940 a, b) consumed as large an amount as 50 mg adrenaline and demonstrated 50—70 % of it partly in combination with sulphuric acid in the urine. According to BEYER and SHAPIRO (1945) adrenaline-like compounds combined with phenols are excreted into the urine.

Author's Researches.

1. Determination of adrenaline injected into the blood.

Although many workers have studied the adrenaline content of peripheral blood by means of chemical methods, and obtained results by them, only quite a few of them have directed their attention to the rapidity with which adrenaline disappears from the circulation. On the other hand, in the use of biological

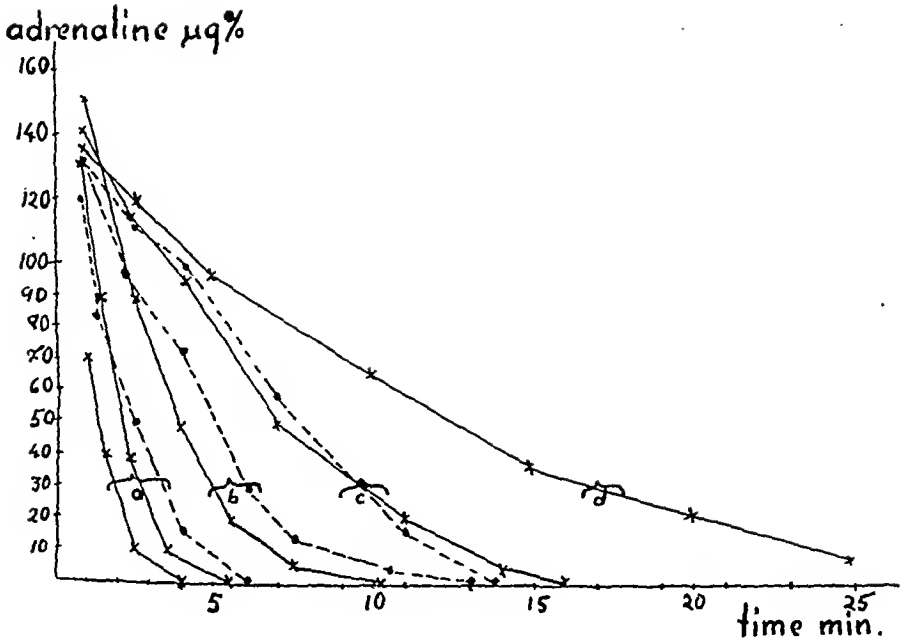

Fig. 37.
Disappearance of adrenaline from the circulation after an injection
——— = *normal test animals*
-------- = *after occlusion of hepatic, renal and intestinal circulation*
a = rabbit, b = dog, c = calf, d = cow.

methods, which have hardly yielded any positive results in adrenaline determinations of peripheral blood, special attention has been paid to the rapid disappearance of adrenaline from the circulation. The tests carried out here by the adsorption method have confirmed the earlier biological tests in demonstrating the disappearance of adrenaline from the circulation.

It should be mentioned again in this connection that adrenaline added to blood is preserved unchanged for several hours and days in blood solution outside blood vessels and does not lose its ability to fluoresce after alkali has been added to the solution.

At first, tests were made to determine adrenaline injected intravenously into the circulation of rabbits. Adrenaline was injected into the ear vein, direct into the heart or into the inferior vena cava. Significant amounts of adrenaline could be determined in samples taken immediately after the injection (fig. 37).

2. Disappearance of adrenaline injected into the circulation.

In samples taken somewhat later after the injection it could be ascertained that there was a complete disappearance of adrenaline already within a few minutes. Six successive test series gave uniform results. After injection of a dose of 100—200 μg adrenaline/kg of body weight, which is nearly lethal, the adrenaline content of samples taken from arteries and veins rapidly decreased already within a few minutes. In several samples it was impossible to demonstrate any adrenaline after five minutes (fig. 37).

In carrying out similar tests on dogs, calves and cows, exactly similar disappearance phenomena could be ascertained. Subsequent to adrenaline injections of 100—200 μg/kg body weight into the circulation of the test animals, adrenaline disappeared from dog blood in about 10 minutes, from calf blood in 15 and from cow blood in 20 to 30 minutes (fig. 37). There were some individual variations in the above results. The experiments were carried out in rabbits and dogs under ether anæsthesia, in calves and cows under evipan anæsthesia. The results also prove that the disappearance is related to the size of the test animal, and thus to the velocity of the circulation.

3. Disappearance of adrenaline from the blood after the occlusion of hepatic, renal and intestinal circulation.

In order to bring to light the factors which have an influence on the disappearance of adrenaline from the circulation, experiments were carried out where part of the circulation was occluded. Adrenaline might either be quickly dialysed into the tissues in the whole capillary area, or disappear in the region of a certain organ. Several workers consider that the liver is the principal factor inactivating adrenaline in the organism, but on the other hand it must be remembered that it is hardly likely for this factor alone to cause such a rapid disappearance of adrenaline. Nor can it be assumed that adrenaline is so rapidly eliminated from the circulation by the kidneys into the urine. In order to elucidate the mechanism of destruction of adrenaline, tests were made where the hepatic, renal and intestinal circulation of the

experimental animals were shut off. In these tests, which were carried out with rabbits, dogs and calves, it could be demonstrated that adrenaline disappeared from the circulation with the same rapidity as was the case with normal test animals. Therefore, these organs alone are not sufficient to bring about a rapid disappearance of adrenaline. Instead these tests evidently point to a disappearance of adrenaline from the circulation into the tissues by means of the whole capillary area of the circulation. This could explain the rapidity of the disappearance of adrenaline. The capillary net is extensive and has a large adsorbing and dialysing surface.

4. The influence of adrenaline-preserving substances on the rapidity of its disappearance from the circulation

According to some investigations ephedrine prolongs the duration of the adrenaline effect in the organism by preventing the destruction of adrenaline by amino-oxydases. On injecting ephedrine together with adrenaline into the rabbit circulation, no difference could be seen in the rapidity of the disappearance of adrenaline.

On injection of adrenaline into the rabbit circulation together with ascorbic acid, which can stabilise the fluorescence reaction of adrenaline and its oxidation *in vitro*, it could not be found that ascorbic acid had any effect on the rapidity of disappearance of adrenaline, or that ascorbic acid *in vivo* could prevent the adrenaline from fluorescing.

Parallel with the disappearance of adrenaline, its effects on the circulation also disappear in test animals, *i.e.* accelerated pulse rate and respiration.

If we take into consideration that fully toxic doses of adrenaline which for a moment enter the circulation, can be eliminated by the organism already within a few minutes, we can well imagine that such amounts of adrenaline as are secreted under physiological circumstances in normal test animals and under the influence of certain stimuli, and which are many times smaller than the former, can also be eliminated by the organism

very rapidly. Evidently adrenaline then disappears at the latest in the capillary area. In peripheral venous blood there is hardly any amount of adrenaline demonstrable by applying the method of fluorescence reaction.

IV

DISAPPEARANCE AND STABILISATION OF ADRENALINE IN THE TISSUES IN VITRO

Earlier Investications.

1. Inactivation.

Different tissues in vitro have a variable effect on the inactivation of adrenaline. Liver mince and extract (LANGLOIS, 1897, LIVON, 1904 a, b, COMESATTI, 1909, SENTYURIN, 1931, KANAUCHI, 1932 a, b, SCHÜTZ, 1933, TOSCANO RICO and MALAFAYA BAPTISTA, 1935 b, BAIN and SUFFOLK, 1936, BORIANI, 1938), intestinal extract (LANGLOIS, 1897, 1898, KANAUCHI, 1932 a, b), arterial wall tissue (TATUM, 1912), pancreatic juice (COMESSATTI, 1909), kidney and lung emulsion (KANAUCHI, 1932 a, b, SCHÜTZ, 1933, TOSCANO RICO and MALAFAYA BAPTISTA, 1935 b) and spleen are able to inactivate adrenaline (TOSCANO RICO and MALAFAYA BAPTISTA, 1935 b), but in muscle emulsion (KANAUCHI, 1932 a, b) and in citrated blood adrenaline disappears very slowly (OLIVER and SCHÄFER, 1895, ELLIOT, 1905, TRENDELENBURG, 1911, 1916, MAIWEG, 1922, SENTYURIN, 1931, KANAUCHI, 1932 a, b, SCHÜTZ, 1933, BROSS and KUBIKOWSKI, 1936, BAIN and SUFFOLK, 1936, BAIN, GAUNT and SUFFOLK, 1937).

The nature of the inactivating agents has also gradually become clear. In the course of the last decade two enzymic factors inactivating adrenaline have been brought to light in the organism. Amine oxidase, which is combined to the amino-group of the side chain of adrenaline, dispersed adrenaline to aldehyde, ammonia, methylamine or ethylamine (HARE, 1928, KOHN, 1937, RICHTER, 1937). According to SCHAPIRA (1945) amine oxidase oxidises adrenaline to phenylethylamine or phenylacetaldehyde. This enzyme is an important oxidising agent in the organism, although its

affinity for adrenaline is low. It is assumed to disintegrate substances related to adrenaline *e.g.* sympathin in postganglionic nerve endplates (GADDUM, 1938). This enzyme was found in all organs with the exception of the suprarenal medulla (SCHAPIRA, 1945). It is present in the liver, intestine and kidneys, in brain and lungs, but not in the heart, muscles or spleen (PUGH and QUASTEL, 1937, BLASCHKO, RICHTER and SCHLOSSMANN, 1937 a, b, c, RICHTER and TINGEY, 1939, LANGEMANN, 1944). It is not solely specific for adrenaline, but also splits other adrenaline-like substances, which are eliminated into the urine as corresponding carbonic acids (EWINS and LAIDLAW, 1910, GUGGENHEIM and LÖFFLER, 1915, RICHTER, 1938, 1940 a, b, FAHRLÄNDER, 1946).

In addition an oxidation of the OH-group of the adrenaline ring and sidechain takes place in the organism (BALL and CHEN, 1933 a, b, KEILIN and HARTREE, 1938, BLASCHKO and SCHLOSSMANN, 1938, 1940). The cytochrome oxidase or phenoloxidase system broke up the adrenaline to a labile adrenochrome, which has 4 hydrogen atoms less than adrenaline and in which the sidechain closed into a five-atom ring. One oxygen atom is sufficient to weaken the effect of the adrenaline molecule on blood pressure. Complete inactivation takes place, when adrenochrome is formed, where two oxygen atom are needed. This oxidation, which is of enzymic nature, proceeds rapidly. Adrenochrome can be further oxidised by 5—6 oxygen atoms through autooxidation to a dark-brown melanin (BLASCHKO and SCHLOSSMANN, 1938, 1940). Adrenochrome forms best at a pH of 6—7 and a temperature of 37°C. An alkaline reaction and a higher temperature catalyse its oxidation to melanin (DEROUAUX, 1943).

Catecholoxidase was found in potato, mushroom and beetle blood (TOSCANO RICO and MALAFAYA BAPTISTA, 1935 c). Tyrosinase from mealworm and mushroom and pyrocatecholoxidase from potatoe oxidises adrenaline in weak solutions first at a pH 5 to adrenochrome and then at a pH 7 to adrenoxine, which has an inhibiting effect on the frog heart and causes a drop in blood pressure (HEIRMANN, 1937 a, b, 1938 a, b, f, 1939, BACQ and HEIRMANN, 1938, HEIRMANN and BACQ, 1938 a, b, e). The same effect is found, when adrenaline is oxidised with oxygen (L'SSAK, 1938 a). Small amounts of adrenaline can cause a vasodilatation (HARPUDER, BYER and STEIN, 1947). The pyrochatecholoxidase was found in all smooth muscle tissue of the vertebrates with the exception of the small intestine (BACQ, 1938 b, c, HEIRMANN, 1938 b, c, d). This explains the inhibiting response of smooth muscles to adrenaline. Adrenoxine is formed also in other tissues (DUCHATEAU-BOSSON and FLORKIN, 1939). Adrenaline is oxidised in the organism also by the indophenoloxidase which is widely distributed in it (HUSZAK, 1932, GREEN and RICHTER, 1937, BHAGVAT and RICHTER, 1938).

In the opinion of PHILPOT and CANTONI (1941) amine oxidase breaks down adrenaline in the liver tissue and the WARBURG-KEILIN system in the heart.

According to GREEN and RICHTER (1937) adrenochrome is an important carrier of hydrogen in the lactic dehydrogenase system, where adrenaline exercises its influence through the coenzyme in still weaker concentrations than usually present in the organism.

In addition to enzymic factors, there are several other substances which are able to oxidise and inactivate adrenaline in the organism. Products of intermediate metabolism of carbohydrate can inactivate adrenaline (BAYER, 1936). Adrenaline acts as a catalyst of the intermediate metabolism, where it is oxidised to quinone (MARQUARDT, 1940 a). Succinic acid, malonic acid and fumaric acid oxidise adrenaline to a red compound, which is pharmacologically inactive (MARQUARDT, 1939 a, b). Adrenaline forms with succinic acid a substance which decreases the blood pressure in the same way as adrenoxine (MARQUARDT, 1939 a, c). After injection of fumaric acid adrenaline solution, succinic acid can increase the blood pressure. Adrenochrome is then reduced back to adrenaline, while succinic acid is oxidised to fumaric acid (MARQUARDT, 1941 b). Adrenaline changes succinic acid to acetaldehyde (MARQUARDT, 1939 a).

Besides substances proper to the organism there are also several agents foreign to it which are able to oxidise and inactivate adrenaline. Oxygen, temperature and pH are important factors in its inactivation (BALL and CHEN, 1933 a, BERRY, WEST and SHOTTON, 1944). The adrenaline is destroyed in physiological Ringer-, Locke- and Tyrode-solutions within a few minutes or hours (OGAWA, 1912, SWETSCHNIKOW, 1914, HARADA, 1925, SUGAWARA, 1928), more rapidly in weak than in strong solutions, but it is more stabile in distilled water. Formaldehyde, too, which is combined to the amino group of adrenaline destroys its physiological activity (ABELOUS and DELAS, 1926, PAGET, 1930 and LEBLOND, 1930, PAGET, LAMELIN and PIED, 1931, TOSCANO RICO and MALAFAYA BAPTISTA, 1935 a). Also quinone, iodine (NOMURA. 1936, OGAWA, 1936, TERAI, 1934 a, b, TERAI and NOMURA, 1935), sodium permanganate (BACQ, 1937 b, 1938 a) and ultra-violet light (KONZETT and WEISS, 1938, 1939, CHATONNET, 1945) destroy it. The oxidation products have a catalytic effect upon its further oxidation (KISCH, 1930). In strong acids the effect of adrenaline also diminishes and totally disappears in basic solutions (SANO, 1933). In thyrotoxicosis and beriberi an injection of adrenaline decreases the blood pressure. This effect can be inhibited by Lugol-solution (AALSMEER, 1932).

The sympathomimetic action of adrenaline is attributable simultaneously to its various chemical groups, benzene ring, phenol group, secondary alcohol group and NH-methyl group (PAGET, LAMELIN and PIED, 1931). The OH-group of the ring and of the sidechain exercises an essential influence on the effect of adrenaline on the intestine and heart (HARTUNG, 1931, YOUMANS, AUMANN and HANEY, 1939, 1940). The most important of them is the meta-OH-group. Oxidised adrenaline increases the tonus of intestine (YEN, 1930).

2. Stabilisation.

Besides destructive factors on the other hand there are substances in the organism which protect adrenaline against oxidation. Adrenaline is preserved in vitro in pulmonary (LANGLOIS, 1897, EMBDEN and FÜRTH, 1904) and muscular tissues (EMBDEN and FÜRTH, 1904, KANAUCHI 1932 a, b, TOSCANO RICO and MALAFAYA BAPTISTA, 1935 b, BAIN and SUFFOLK, 1936) as well as in blood (OLIVER and SCHÄFER, 1895, ELLIOT, 1905, TRENDELENBURG, 1911, 1916, MAIWEG, 1922, SENTYURIN, 1931, KANAUCHI, 1932 a, b, SCHÜTZ, 1933, BROSS and KUBIKOWSKI, 1936, BAIN and SUFFOLK, 1936). In the vitreous humor, lymph (BONHOMME, 1936), pericardium fluid (BÂLTÂCEANU, VASILIUS and NOVAC, 1936), uterine extract (LISSAK, 1938 a), heart extract (SMITH, 1937) and in protein solutions (DONINI, 1937, 1938) the colloidal substances with large molecules protect adrenaline against oxidation. In addition, the brain, liver and thymus lipides prevent the oxidation of adrenaline (KIRALY, 1942). In the suprarenal medulla the breaking-up of adrenaline is prevented by ascorbic acid and glutathione, of which there is more in these glands than in other tissues (v. SZENT-GYÖRGYI, 1930, v. EULER and KLUSSMANN, 1933, HUSZAK, 1933, BINET, 1933, BERSIN, KÖSTER and JUSATZ, 1935, DEUTSCH and SCHLAPP, 1935, GUHA, CHATTERJI, DAS and GHOSH, 1935, KUCHEL and MITCHELL, 1936, KOTSCHNEW, KRYSHANOWSKAJA, LONDON and RIWOSCH, 1938). The perfusion fluid of the suprarenal glands also protects adrenaline against oxidation (HEARD and WELCH, 1935). GUHA (1935), DAOUD and AYYADI (1938) considered that ascorbic acid did not heighten the effect of adrenaline. In scurvy adrenaline does not increase blood pressure and blood sugar (MARUI and MACHII, 1932, KASAHARA, NISHIZAWA and HIRAO, 1937). Vitamin P also inhibits the oxidation of adrenaline (LAVOLLAY and NEUMANN, 1941, PARROT and COTEREAU, 1946).

The principal substances with small molecules preventing the oxidation of adrenaline in the body are ascorbic acid, cysteine, glutathione, guanidine and amino acids (ABDERHALDEN and GELLHORN, 1923, 1924, WILTSHIRE, 1931, HEARD and WELCH, 1935, WELCH, 1934, VIALE, 1934 a, b, ABDERHALDEN, 1934, 1936, BURNS and SECKER, 1936, IWO, 1936 a, b, TAUBER, 1936, YAMAMOTO, 1936, BLASCHKO, RICHTER and SCHLOSSMANN, 1937 d, MARQUARDT, 1938, 1940 b, 1941 c, STANISTREET and BYRNE, 1946). The negative potential of ascorbic acid protects adrenaline against oxidation in the organism (BALL, 1937). Adrenaline-like substances are found in cells in a reduced state (BALL and CHEN, 1933 a).

Importans agents stabilising adrenaline are also low pH, low temperature and many inorganic and organic substances, e.g. acids (VACEK, 1927, carbon dioxide (SCHMUCK, GAWRILOW and KRASILNIKOW, 1932, ROWLINSON and UNDERHILL, 1939, v. ESVELD and v. GENDEREN, 1942), potassium and sodium metabisulphite (SJÖGREN, 1935. WEST,

1945 a, b, 1946 a, b), same metallic ions, phenols, barbituric acid, substances containing the NH-group (BAUR and OBRECHT, 1938) or SH-group (LILY, 1936) and benzoic acid (DONATELLI, 1940). According to MÖLLER and SCHOU (1942) sulphite does not increase the preservability of adrenaline, but only prevents adrenaline from being coloured. Adrenaline is also preserved in the redox system which is formed by an inorganic colour substance and its leuco-compound, e.g. methylene blue and its leuco-form (NORDMARK-WERKE, 1937). Adrenaline catalyzes the reduction of methylene blue in the presence of glyceric aldehyde (SEITZ, 1939).

There are substances, which not only stabilise adrenaline, but are also able to transform inactivated adrenaline back to its effective stage (BACQ, 1935 b, BARCIA, 1946 b). When adrenaline has been oxidised to an inactive quinone, the post-ganglionic sympathetic nerve fibres reduce it back to an active phenol. The oxidation products of adrenaline, carried to the nerve fibres by way of the blood, would thus be transformed in the organism to sympathin (BACQ, 1935 b). The isolated toad heart excited to exhaustion can respond again after an addition of adrenaline completely oxidised by phenolase or by exposured to air. The sympathetic nerve endings have then formed adrenaline from the oxidised adrenaline (BARCIA, 1946 a, b). Adrenaline inactivated in vitro by quinone, iodine or tyrosinase, in the same way can be restored by hydroquinone (TERAI, 1934 a, CAMP, 1936, AIHARA, 1936), phenyleneamine (NOMURA, 1936), several amino acids (TERAI and NOMURA, 1935), ascorbic acid (TAUBER, 1936), homogentisic acid (AIHARA, 1935, OKAGAWA and ICHITSUBO, 1935) and sodium sulphite (OGAWA, 1936, OKAGAWA and ICHITSUBO, 1935). Ovariectomy (KARASEK and POUPA, 1938) and splenectomy (ROMEL, 1940) can reduce the vascular sensitivity to adrenaline. After ovariectomy the injection of folliculin or testosterone augments the action of adrenaline.

Some substances not only stabilise, but also enhance the physiological effect of adrenaline, e.g. amino acids and polypeptides (ABDERHALDEN and VLASSOPOULOS, 1930, HORIMI, IWO and OTO, 1937, LEVI, 1937, ISIHARA, YORIMITU, OTODA and HUSE, 1938, SATAKE, 1938, WEBER, 1939, FONTAINE, HUCHE and LE BRETON, 1943), blood serum and organ extracts (HATTORI, 1938, MYLON and HELLER, 1947, MYLON, HORTON and LEVY, 1947 a, b), thyroid hormone (TADA, 1930, HORIMI, 1936), acetylcholine (DANIELOPOLU and MARCOU, 1940, McDOWALL, 1947), aneurin (GEROKARNI, 1942, PETRILLO, 1946), ascorbic acid (KREITMAIR, 1934, AIHARA, 1936, KASAHARA, NISHIZAWA and HIRAO, 1937, NOLTE, 1938, SHIMAMURA, 1938, NUKIDA, 1939, GEROKARNI, 1942, MARQUARDT, 1943, CIRERA, 1946, MEIDINGER, 1946), nicotinic acid (GÖBELL, 1940), pyrogallol (KREITMAIR, 1934, BACQ, 1936, KASAHARA and KAWAMURA, 1937, KASAHARA, NISHIZAWA and HIRAO, 1937) and hexamethylentetraminjodmethylat (KASWIN, 1939). The effect of adrenaline increases also in weak acid (SANO, 1933) and in cold

(BONSIGNORE and LOMBROSO, 1938). Oxygen can prolong the effect of adrenaline on the intestine (STARKENSTEIN, 1940), but in starvation and phosphorus poisoning the organism reacts more sensitively to adrenaline (MARUI and MACHII, 1932).

Some substances can prolong the effect of adrenaline in the organism by inhibiting its enzymic splitting. The amine oxidase is impeded by *e.g.* α-metylated amines, ephedrine and benzedrine (GADDUM and KWIATKOWSKI, 1938, BLASCHKO, 1940), cocaine (FRÖHLICH and LOEWI, 1910, SAKUSSOW, 1931, SUZUKI, 1939, PHILPOT, 1940), sodium salicylate (BORIANI, 1938) and vessel-constricting imidazolidine (SCHARWÜTHRICH, 1943). On the other hand, ortophenolases are impeded by substances containing the SH-group and by ascorbic acid (TOSCANO RICO and MALAFAYA BAPTISTA, 1935 d, CIRERA, 1946). The inactivating effect of succinic acid and acetaldehyde in organ mince can be impeded by cocaine, sparteine (BAYER and WENSE, 1938 a, b, WENSE, 1939 a, b), ascorbic acid and malonic acid (MARQUARDT, 1938, 1941 a. c).

Author's Researches.

1. Tissues' own fluorescence.

Several tissues contain considerable amounts of fluorescing substances which are dialysed through the cellophane membrane and disturb the determination of small quantities of adrenaline in the dialysing fluid. Especially when dialysing mince prepared from liver tissues, the cellophane membrane is passed by components with a strong yellow fluorescence, whose strength and colour vary at different pH-values. Substances with a blue fluorescence are present to some extent in almost all tissues.

2. Dialysis of adrenaline added to the tissues.

If adrenaline is added to liver or liver extract and thereafter a dialysis of adrenaline against distilled water is carried out, it is found that not even significant additions of adrenaline are able to produce the fluorescence reaction typical of adrenaline after the addition of alkali. Yet it was possible in this case to demonstrate in the dialysing fluid the red oxidation product of adrenaline with the help of v. EULER's iodine oxidation reaction (1933 b), which proved that adrenaline was dialysed from the liver

tissues in the expected amount. The same property inhibiting the fluorescence reaction is also found in several glandular tissues, although to a smaller extent than in the liver. To these belong the thyroid gland, pancreas, kidney, spleen, and ovaries. On the other hand, the fluorescence reaction of adrenaline is obtained almost as well from muscular tissue and heart muscle as from blood.

3. Elimination of factors preventing the fluorescence reaction of adrenaline in tissues.

If in a dialysing fluid where the fluorescence reaction of adrenaline fails to appear on addition of alkali, an oxidation of adrenaline to the red oxidation product is first carried out by means of v. EULER's iodine reaction (1933 b), it is found that this oxidation product causes on addition of alkali the fluorescence reaction typical to adrenaline. The iodine oxidation reaction has oxidised adrenaline to such a state that the fluorescence reaction occurs on addition of alkali. It is evident that substances preventing the oxidation of adrenaline have become dialysed from the tissues. By means of a dichlorophenolindophenol titration it is possible to find a greater amount of reducing agents in just those tissues, especially in liver, kidneys, thyroid gland, pancreas, spleen and ovaries, where the fluorescence reaction of adrenaline is most strongly inhibited, whereas there are lesser amounts of reducing agents in muscles and blood, where it is easy to make the adrenaline fluorescence reaction appear. Thus the tissues have an evident ability to prevent an oxidation of adrenaline with the help of reducing substances to a state giving fluorescence reaction.

Summary.

Attempts have been made to throw some light on the chemical determination of adrenaline, its occurrence and metabolism in the animal organism. With the help of the fluorescence reaction of adrenaline in alkaline solutions endeavours have been made to develop a method for the determination of adrenaline of as great a sensitivity as lies within the limits of possibility.

At the beginning of this study attention is drawn to those factors which can influence the progress of the fluorescence reaction, in order to demonstrate the possibilities of quantitative determinations of adrenaline by utilising the fluorescence reaction. The adrenaline contents, temperature, pH, oxidising and reducing substances present in the solution, are set forth as factors influencing the fluorescence reaction.

The appearance and disappearance of the fluorescence reaction is delayed or prevented by a low pH, low temperature and reducing substances.

The appearance and disappearance of the fluorescence reaction is promoted by a strongly alkaline reaction, high temperature and oxidising agents.

The delaying and catalysing factors have an antagonistic effect; delaying factors can stabilise adrenaline against a rapid oxidation and promoting factors can catalyse the delayed oxidation. The fluorescence reaction appearing in adrenaline solutions can fail to become visible under the influence of both these factors.

Since the determination of small quantities of adrenaline is disturbed by several by-fluorescences, attention has been paid

to the elimination of by-fluorescences from the dialysis dishes, the cellophane membrane and the rubber layer.

By means of the dialysis methods studies have been made of the dialysis of adrenaline from blood and from water solutions, of the preservability during the dialysis, as well as of the effect of pH, type of membrane and aluminium hydroxide.

The dialysis experiments have shown that adrenaline is dialysed from aqueous solutions to the equilibrium, from blood solutions 20 % less. Aluminium hydroxide is proved to increase the dialysis of adrenaline from blood solutions by means of an adrenaline adsorption.

By studying the adsorption of adrenaline and its persistence in the recipient fluid in various pH-zones after dialysis from blood a procedure has been developed in which, to prevent the adsorption of adrenaline to aluminium hydroxide, the reaction of the blood to be dialysed was made less basic by an addition of acid.

On the other hand, a procedure has been developed in which adrenaline is adsorbed to aluminium hydroxide by arranging conditions suitable for adsorption. The dialysis is carried out here from whole citrated blood against a corresponding volume of distilled water to which aluminium hydroxide has been added for the adsorption of adrenaline. In the process of dialysis the pH of the dialysing fluid then rises to about 8.5, in which reaction the adrenaline is adsorbed to aluminium hydroxide. The adrenaline is determined after centrifuging from the aluminium hydroxide precipitate with the help of the fluorescence reaction by means of adding alkali.

By making use of the adsorption of adrenaline to aluminium hydroxide the sensitivity of the dialysis method can be increased 6 to 7 times. The adsorption method makes it possible to determine the adrenaline content in the blood even at 0.5—1 μg %.

By utilising the adsorption method, tests have been made for a determination of the adrenaline in peripheral and central blood in man and in test animals. A clearly demonstrable adrenaline content could be found only in the blood of the suprarenal vein, and in the blood of the inferior vena cava provided the adrenaline

secretion was increased by injecting insulin or morphine. Rabbits, dogs, cows and calves were used as experimental animals.

Using rabbits, dogs and calves as test animals it is shown that adrenaline very rapidly disappears from the circulation. Even if significant amounts of adrenaline are injected into the circulation of the test animals, adrenaline is rapidly adsorbed to the tissues, evidently in the capillary area, the time being 5 minutes for rabbit circulation, 10 for dog, 15 for calf and 20—35 minutes for cow.

In order to elucidate those factors which influence the rapid disappearance of adrenaline from the circulation, experiments have been made with occluding the circulation of some organs in test animals for the time of the experiment. This has brought to light the fact that the occlusion of the renal, hepatic or intestinal circulation has no effect on the velocity of the disappearance of adrenaline. Nor did the tests show that substances which protect adrenaline from dispersion, *i.e.* ephedrine and ascorbic acid, could prevent the rapid disappearance of adrenaline. Since even very large amounts of adrenaline quickly disappear from the circulation, it becomes evident that adrenaline is rapidly dialysed into the tissues in the entire capillary area, whereafter a further splitting takes place.

In order to explain the phenomenon of the disappearance of adrenaline from the circulation experiments have been made to demonstrate adrenaline in the tissues. It was found that the fluorescence reaction of adrenaline was strongly impeded in several tissues. Adrenaline added to mince prepared from liver, kidney, brain, pancreas, thyroid gland and spleen does not yield any fluorescence reaction in the dialysing fluid. On the other hand, adrenaline added to minced tissue prepared from muscle and heart muscle can be demonstrated after dialysis almost equally well as in blood.

In tissues where the adrenaline fluorescence reaction is inhibited it can be made to appear by oxidising the tissue reducing agents with iodine. The prevention of the fluorescence reaction of adrenaline in the tissues runs parallel with the content of reducing substances appearing in them.

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ON THE FUNCTIONAL ORGANIZATION OF THE MOTONEURONS IN THE SPINAL CORD

by

Karl Erik Åström

Stockholm 1948

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Preface.

The investigations to be described in this work were carried out at the Physiological Department, Karolinska Institutet, Stockholm 1947—1948. This publication provides me with the opportunity to express my deepest thanks to Professor Carl Gustaf Bernhard, who has always most unselfishly facilitated my work by giving me valuable advice, keen criticism and stimulating encouragement. I am also deeply indebted to Docent Bror Rexed, who has given me many useful instructions concerning the histological problems in this work. My thanks are due to Mrs. Anna Moberg, who has drawn most of the figures, and to Miss Kate Nilsson for valuable assistance. Finally I desire to express my best thanks to Miss Dorothy Ferris for the care she has taken with the translation of this work.

Stockholm, October, 1948.

Karl Erik Åström.

Introduction and Problem.

Increased knowledge has been acquired in the course of later years concerning the functional properties and localization of the central neurons by using microelectrodes for local activation within the central nervous system. Thus, in a series of investigations, in which the microstimulation of motoneurons and premotor tracts was carried out in the brain stem, LORENTE DE NÓ investigated the excitability of the soma of the motoneurons (1935 b—f), the anatomical and functional basis for the synaptic transmission (1935 a, 1938 a, b, 1939) and a fundamental analysis of the organization of the chains of internuncial neurons (1938 c). Further, LLOYD analyzed in a series of investigations the functional organization of the pyramidal, bulbospinal and propriospinal tracts (1941 a and b, 1942, 1944) by using microelectrodes for activating and recording within the brain stem and the spinal cord.

RENSHAW (1940) found that stimulation of the grey matter of the spinal cord with a microelectrode elicits two waves in the corresponding ventral root. The first, which is due to direct stimulation of the motoneurons, is most easily evoked from the ventral part of the cord, while the second, which is caused by the activation of premotor elements (the relayed volley), is more easily elicited when the point of the electrode is placed more dorsally in the cord. By adopting a similar technique for activation combined with subsequent histological control of the position of the electrode in the spinal cord, BERNHARD and REXED (1945) found that volleys relayed over one synapse from the segments L4—S1 of the spinal cord were most easily elicited when the point of the electrode was placed in the lateral part of the intermediate zone. They concluded that this area contains association neurons discharging to motoneurons, which activate the leg muscles.

In a number of earlier papers on the reciprocal innervation the need of increased knowledge concerning the intraspinal distribution of the motoneurons has been pointed out (see *e. g.* SKOGLUND 1946, 1947, BERNHARD and THERMAN 1947, BERNHARD 1947). The theme of this work is an analysis of the intraspinal parts of the motoneurons innervating *m. gastrocnemius* and its antagonists *m. tibialis anterior* and *m. extensor digitorum longus* by local activation within the spinal cord with the help of a microelectrode.

In a preliminary report (ÅSTRÖM 1947) it was suggested that local stimulation of different intraspinal structures of the motoneurons is possible, and that this fact can serve as a basis for an analysis of the intraspinal organization of different motoneuron systems. The experiments described in this work have been carried out on this basis.

Anatomical Review.

The motoneurons, the axons of which run to the voluntary muscles of the trunk and the limbs, have large cell bodies (transversal diameter maximally 60 μ and axial diameter maximally 100μ in man according to LHERMITTE and KRAUS, 1925). The cell bodies are situated solely in the anterior columns of the grey matter (*cf.* CAJAL's discussion 1909, p. 354 concerning other opinions). One axon and numerous dendrites branch off from each cell body (CAJAL 1909). The axons collect in bundles, which leave the spinal cord on the same side and level in which the corresponding cell bodies are situated, in order to form the filaments of the ventral roots (SHERRINGTON 1892, BOK 1928).

In cross sections of the cord the cell bodies are seen to form groups, which are more numerous and differentiated in higher than in lower vertebrates (KAPPERS 1920). In lengthwise sections they appear as columns of different lengths, these columns having no direct relation to the segment boundaries indicated by the spinal roots (ELLIOTT 1942).

According to generally accepted terminology (BRUCE 1901 quoted from RANSON and CLARK 1947) the cell columns in highly developed vertebrates are divided into two categories, lateral and medial. The medial columns are to be found along the whole length of the spinal cord, while the lateral, divided into the anterolateral, posterolateral, retroposterolateral and central columns, are to be found only in the enlargements of the spinal cord. The lateral groups are slightly differentiated within the cervical or lumbar enlargement in animals with poorly developed fore or hind limbs (KAPPERS 1920). There are also considerable variations in the configuration of the cell groups in the different species of mammalia with well developed extremities (man, ONUF 1899, BRUCE 1901, PARHON and GOLDSTEIN 1905, JACOBSOHN 1908, ELLIOTT 1942, 1943; gorillas, WALDEYER 1889; other apes, BIKELES

and FRANKE 1905, REED 1940; dog, DE NEEF 1900, MARINESCO 1904; albino rat, ANGULO Y GONZALES 1927, 1940; rabbit, ROMANES 1941 and cat, KIESEL 1938, ELLIOTT 1944, REXED 1948).

ELLIOTT (1944) compared the grouping of the motor cell bodies in man, primates, dogs, cats and rodents. He found that the grouping of cell bodies in the full grown cat is similar to that in man.

ANGULO Y GONZALES (1940) and ROMANES (1941) have shown that cell bodies of the motoneurons segregate from the originally undifferentiated cell masses in the ventral part of the mantle layer in a regular order. The differentiation takes a cephalocaudal direction. The groups of cell bodies are first formed in the medioventral part and the differentiation then continues in a laterodorsal direction along the boundary line between the anterior column and the white matter. Hence, the caudal and dorsal parts of the lateral groups are the last to be formed.

It is generally supposed that the comparatively constant grouping of motor cell bodies in the different species implies that the cell groups may be correlated in some way or other to the spinal nerves or to the voluntary muscles, *i. e.* that the grouping of the cell bodies reflects the periphery. There are, however, different opinions concerning the definition of "the periphery". Most authors agree that the motor nerve fibres from the medial groups innervate the trunk musculature, and those from the lateral groups run to the limbs (see *e. g.* BOK 1928). There is also general agreement in favour of the opinion that among the lateral cells the ventral groups correspond to the proximal part and the dorsal to the distal part of the limb (SHERRINGTON 1892, MARINESCO 1904, ROMANES 1941). For the rest the opinions differ considerably, however, as to the principles according to which the cell bodies and the peripheral organs are correlated. It has been suggested that the cell groups may correspond to: 1) separate muscles (SANO 1904, MARINESCO 1904), 2) groups of muscles (ONUF 1899), 3) parts of an extremity, *e. g.* the thigh, lower leg, foot (VAN GEHUCHTEN and DE BUCK 1898, ROMANES 1941), 4) muscles with similar functions (COLLINS 1894, GOERING 1928), 5) embryonic myotomes (BOK 1928).

According to STREETER (1911), DETWILER (1936) *et al.* the peripheral motor nerves are secondarily formed under the influence of developing musculature. Further, it has been shown that the cells in the mantle layer are segregated in groups parallel with

the development of the limbs (ANGULO Y GONZALES 1940, ROMANES 1941). From these observations it is probable that the cell groups are influenced by the development of the musculature during the ontogenesis. It has not been possible, however, to show whether the muscles of the extremities in mammals develop straight from the mesodermal tissue in the limb buds or whether they are of myotomic *i. e.* segmental origin (see LEWIS 1910, HAMILTON, BOYD and MOSSMAN 1945). In this connection it should also be mentioned that according to STREETER (1933) there is no true segmental arrangement inside the spinal cord during the ontogenesis. DETWILER (1936) likewisely states "that for the urodeles at least, the segmentation of the spinal cord and peripheral nerves is entirely subservient to mesodermic metamerism and that all intrinsic nervous segmentation is non-existent".

SHERRINGTON's well-known work on the arrangement of motor fibres in the lumbo-sacral plexus (1892) deals with the extra medullary course of the motoneurons. It provides, though indirect, valuable information as to the position of the motoneurons belonging to different nerves. He showed that the axons to each of the muscles of the hind leg leave the spinal cord by ventral root filaments lying in consecutive order. The regions for the outflow of efferent fibres to the leg muscles will always be found in the same position in relation to each other in different animals belonging to the same species. They may, however, lie more cranially (prefixation) or caudally (postfixation) in relation to the segmental levels indicated by the spinal roots.

Since the motor axons leave the spinal cord on the level on which their cell bodies are situated (cf. SHERRINGTON 1892 and BOK 1928), it can be assumed that the region from which a muscle or a group of muscles receives motor fibres corresponds to a column of cells which is situated on a level corresponding to the outflow of these motor fibres.

According to SHERRINGTON (1892) the motor nerve fibres to the deep peroneal and the gastrocnemius nerves (*i. e.* the nerves to be investigated in this work) leave the spinal cord in the cat mainly at segment L6—L7 and L7—S1 respectively. Hence, the columns for the cell bodies of these nerves overlap within at least one segment (L7).

The investigations referred to, however, do not deal with the dendrites, which are particularly well developed formations in the motoneurons. They are long, ramified formations, which,

according to CAJAL (1909), can be divided into commissural, anterior, marginal, posterior and longitudinal dendrites. They are more numerous and more developed in lower vertebrates than in mammals (KAPPERS 1920). Our knowledge as to their structure, position and qualities, however, is incomplete so far, as pointed out by *e. g.* LORENTE DE NÓ (1938 b).

Technique and Procedure.

Most of the experiments were performed on cats under dial anesthesia (0.4—0.6 cc 10 per cent, Dial, Ciba per kg). During the long experiments (15—20 hours) it was often necessary to inject another 0.3 cc dial. In a few experiments decerebrated or spinal cats were used. The results from these preparations are in good agreement with those under dial anesthesia.

After lumbar laminectomy the dorsal roots were cut bilaterally from L5 to S2. In both hind legs the branches from the tibial nerve to the two heads of *m. gastrocnemius* (below called the gastrocnemius nerve) and the branches to *m. tibialis anterior* and *m. extensor digitorum longus* (below called the deep peroneal nerve) were prepared. These nerves have reciprocal functions, *i. e.* extension and flexion in the ankle joint (see SHERRINGTON 1910, LLOYD 1943).

In order to avoid muscular contractions when the spinal cord was stimulated, the femoral and obturator nerves, the hamstring, the rest of the tibial and the peroneal nerves were cut as also the tendon of *m. ileopsoas*.

The animal was placed in a warm, screened box and fixed with clamps to the fourth spinal process and the hip bones. The exposed part of the spinal cord and the nerves used were covered with paraffin oil.

A fine needle electrode, which was insulated except for the point, was used for the microstimulation of the spinal cord. The diameter of the non-insulated tip was about 0.03 mm. The electrode was controlled microscopically both before and after each experiment, and it was discarded as soon as there was any visible sign of the insulation getting worn. The indifferent electrode was clamped to a spinous process. The electrical stimulus to the spinal cord was a square wave (0.1—0.4 msec. duration) obtained from a stimulator, the shocks being transmitted over transformers. The

stimulating current was led to one of the beams of a double cathode ray oscillograph, and the strength of the stimulus could be read in arbitrary units. The resistance via the microelectrode and over the preparation was measured in a series of experiments, when measuring from different points it was found to be about 60,000 ohms. Variations in the resistance being insignificant, proportionality exists between the size of the deflection, which marks the stimulus on the cathode ray beam, and the strength of the stimulating current.

The action potentials were recorded from the deep peroneal and the gastrocnemius nerves on both sides with the aid of a condensor-coupled amplifier (time constant 30 msec.) and the cathode ray oscillograph.

The experiments were carried out by stimulation within the seventh lumbar segment of the spinal cord, this segment being the only one which usually contains the cell bodies belonging to the two nerves mentioned (SHERRINGTON 1892).

The threshold values for the action potentials due to stimulation of the motoneurons (below called direct volleys or direct waves) in the different nerves were determined at varying positions of the stimulating point, the needle being pushed through the spinal cord with the aid of a micrometer gauge.

Figs. 1 and 2 show some records illustrating the potential response in the deep peroneal nerve to stimulation by the needle in the L7 segment of the spinal cord with increasing stimulus strength (A—C). With the needle point in a position as in the experiment illustrated in Fig. 1 only one potential wave was obtained in the gastrocnemius nerve. The latency of this wave is 1.9 msec., which shows that only postsynaptic elements, *i. e.* the motoneurons, were stimulated since, this latency corresponds to the conduction time in the peripheral nerves with highest conduction velocity (cf. LLOYD 1943). With the needle point in a position as in Fig. 2 both presynaptic and postsynaptic elements are stimulated as shown by the two waves, the second one (the relayed wave) in this case having lower threshold. The difference in the latencies for the two waves is 0.9 msec. which represents the synaptic delay (cf. RENSHAW 1940, BERNHARD and REXED 1945). This work only deals with the direct volleys, the latencies being controlled in each experiment.

The latencies for the direct volleys evoked from the dorsal part of the cord are 0.2—0.3 msec. longer than the latencies

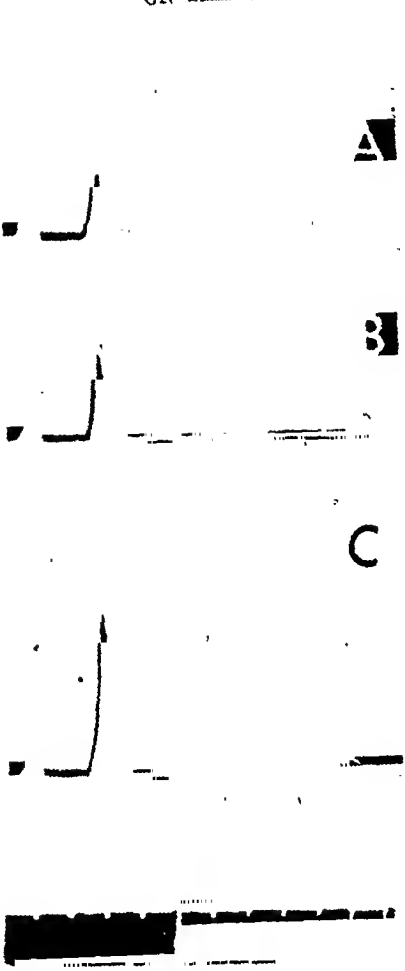

Fig. 1 A—C. Direct potential wave in the deep peroneal nerve to stimulation by needle electrode in the middle part of the grey matter of the seventh lumbar segment of the spinal cord. Increasing stimulus strength downwards (A—C). Time in msec.

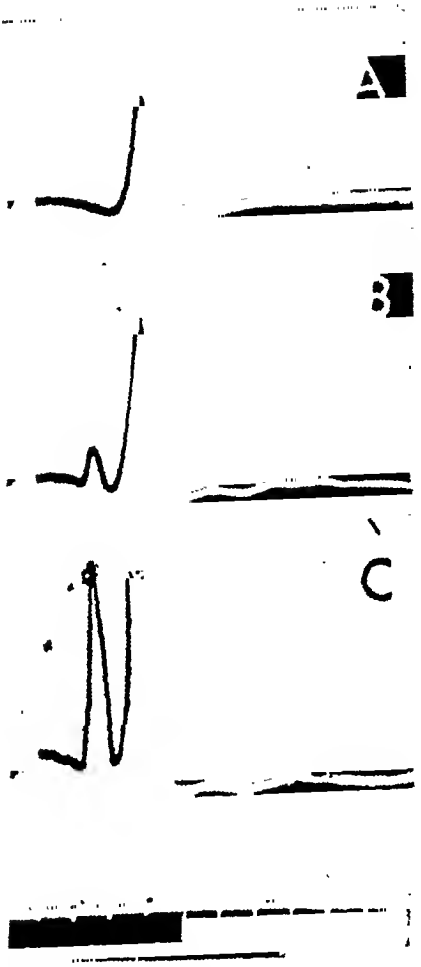

Fig. 2 A—C. Direct and relayed potential waves in the deep peroneal nerve to stimulation in the ventral part of the lateral funiculus of the seventh lumbar segment of the spinal cord. The second volley (the relayed one) has a lower threshold than the direct wave. Increasing stimulus strength downwards (A—C). Time in msec.

for those from the ventral part. Further, the latencies for the direct waves recorded from the contralateral nerves (in relation to the position of the stimulating needle) are longer (0.1—0.3 msec.) than the latencies for those from the ipsilateral nerves.

On completion of the experiments the animal was killed. The heart was opened and a cannula was put into the aorta from the left ventricle. The vascular system was washed with Ringer's solution and then with 10 per cent formaldehyde, the tissues being fixed in situ. The part of the spinal cord caudal to the L4 segment

was removed carefully. This piece of the cord was frozen and microscopical studies made of serial unstained sections (100 μ thick).[1]

The traces left by the needle electrode were very distinct in the shape of a channel which was often filled with red blood corpuscles. As the sections were seldom cut parallel with the channel, it was not possible to see more than a part of it in each preparation. Hence the channel had to be reconstructed by examining a series of sections. The pictures of the cross sections have been drawn from the microscopic preparations on millimeter paper with the help of a micrometer ocular. 20 mm in the drawings correspond to 1 mm in the sections.

Altogether 97 experiments were carried out on 44 cats, the most representative results being given below. Every experiment included a series of stimulations with successive shiftings of the needle from the dorsal to the ventral surface.

At the beginning of the experiment the point of the needle was placed on the surface of the spinal cord and was then pushed ventrally. The threshold value for the direct volley in the four different nerves was read for each position. The relation between the positions of the point of the needle and the corresponding threshold values is given in the diagrams, where the values of the stimulus strength in arbitrary units (vertical axis, logarithmic scale) is plotted against the distance of the point of the needle from the dorsal surface (horizontal axis, linear scale). Fig. 4 is an example of such a curve. As will be seen, it is made up of a high, a falling and a low part. The lowest thresholds are obtained when the ventral part of the cord is activated (cf. RENSHAW 1940, BERNHARD and REXED 1945). This is also to be expected, since this part contains the cell bodies of the motoneurons and axons. The threshold values on the falling part of the curve to the left of arrow 1 might then represent activation due to the current being spread to motor elements situated ventral to the area stimulated. The following, however, will show that the curves assume a different form from that in Fig. 4, at different positions of the electrode, and that the differences between the curves imply local activation of elements situated within other regions than in the anterior column (see *e. g.* Fig. 15 which illustrates activation of the anterior commissure). Further it will be shown (see page 24) that the spread of current round the point of the needle is so slight

[1] The histological technique proposed by Docent B. REXED.

that the curve in Fig. 4 would have been steeper than it actually is, if the threshold values had represented activation of motor elements situated ventral to the point of the electrode. Thus, the values of the curves represent the threshold values of the motor elements in the immediate vicinity of the point of the needle. It must be pointed out, however, that the threshold values in this case are not only a measurement of the excitability of the axons, the dendrites or of the cell bodies, but they give also information about the density of the motor elements round the point of the needle. In view of this it is now possible to draw the following general conclusions from the curve in Fig. 4. The high part shows the existence of sparse elements with low excitability, the low part shows elements densely collected together with high excitability, while the falling part corresponds to an area containing elements with increasing density and possibly even increasing excitability. It must be emphasized that the threshold curves give no information as to the absolute density or excitability of the elements.

The threshold values are indicated in logarithmic units (vertical axis), changes in the low values of the curves being of comparatively greater importance than is the case in the high ones.

As will be seen from the figures, the length of the needle channel in the cross section is shorter than the actual displacement of the needle point (*i. e.* on the horizontal axis of the diagram from point 0 to the terminal of the curve). This difference is certainly not due to shrinkage, since it is known that 10 per cent formaldehyde solution does not change the volume of the nerves (REXED 1944). Instead special experiments have shown that this discrepancy is primarily due to the fact that the surface of the spinal cord, which is made up of pial and subpial tissue, bulges under the microelectrode when the needle perforates the surface, and that this bulge still remains when the needle is moved ventrally. Thus, this error, which amounts to 1.0—1.5 mm., is to be found near the zero point on the horizontal axis. Hence, in order to determine the position of a point with a certain threshold value, the distance from the terminating points of the curve and the needle channel has been used. No conclusions have ever been drawn from the first part of the curves (1.0—1.5 mm. from the zero point on the horizontal axis). Further, it has been shown that the resistance was considerably lower when the point of the electrode was placed on the dorsal surface of the cord than it was when the needle penetrated it. This

fact explains the rising phase, which is sometimes found in the first part of the curves (see *e. g.* Fig. 5).

The needle channel sometimes assumes a crooked course with its convexity directed ventromedially (see *e. g.* Fig. 6). This takes place when the needle goes in a ventral or ventrolateral direction through the lateral part of the spinal cord, and is due to the spinal cord turning round its longitudinal axis owing to pressure of the needle.

Each experiment comprises readings from all the four nerves. In order to simplify the diagrams, however, only one or two threshold curves are given in each.

Results.

Local Activation of the Intraspinal Parts of the Motoneurons Innervating the Gastrocnemius Muscle.

a) Cell Bodies and Axons.

Fig. 3 gives the threshold curve for the direct volleys in the gastrocnemius nerve from an experiment in which the electrode passed through the posterior and anterior columns of the spinal cord in a ventromedial direction. The lowest threshold value is to be found at the end of the curve. The cross section shows that this value (arrow 1) corresponds to the stimulation of a point near the border between the grey and the white matter close to one of the bundles of motor nerve fibres, which runs through the anterior funiculus towards the ventral surface of the spinal cord. It is obvious that the point of the needle stimulated dense lying elements with high excitability. The form of the curve gives no immediate information as to which intraspinal parts of the motoneurons were excited. There are two possibilities, *viz.* that the needle approached and then fell tangent to or perforated either 1) cell bodies or 2) a bundle of axons. The first alternative is the less likely of the two, since low theshold values as seen in Fig. 3 are never found when a group of motor cell bodies is stimulated locally (see p. 24). It is more probable that the last part of the curve in Fig. 3 represents the stimulation of a bundle of axons, which may have their origin either in medial or lateral motor cell bodies (according to the previously mentioned terminology). If the needle point had stimulated the axons from the medial group, it would have crossed these fibres a little bit from the end of the channel. Obviously this is not the case, since the threshold curve would then have a horizontal or

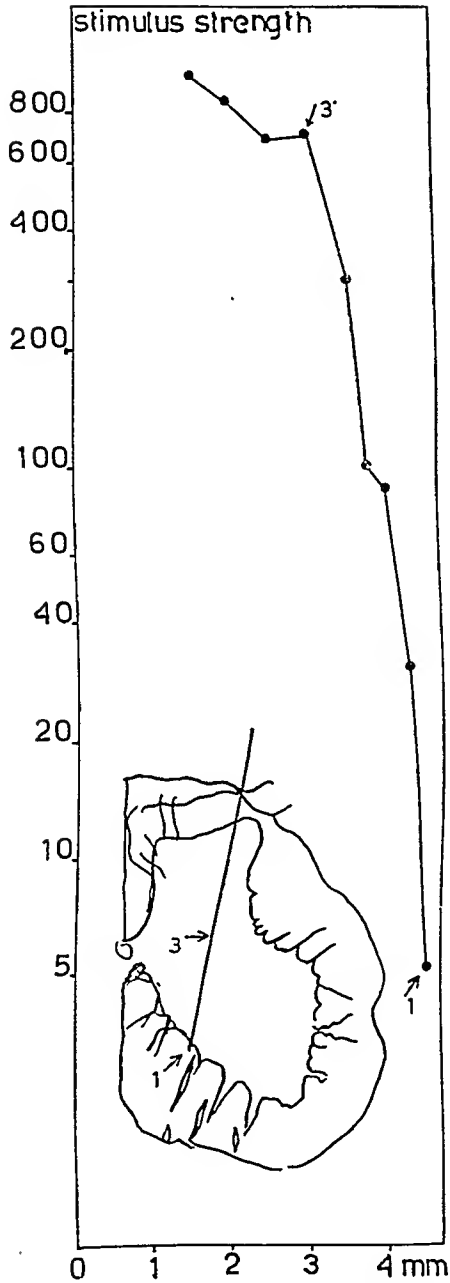

Fig. 3. Threshold curve for direct volleys in the gastrocnemius nerve. The strength of the threshold stimulus in arbitrary units (vertical axis, logarithmic scale) is plotted against the distance between the point of the needle and the dorsal surface of the spinal cord in mm (horizontal axis, linear scale). Microstimulation in the middle of the seventh lumbar segment of the cord. The inset shows the position of the channel made by the needle electrode, the figure being microscopically reconstructed from serial sections. The scale (in mm) is the same as the one on the horizontal axis of the diagram. For interpretation of the curve see text.

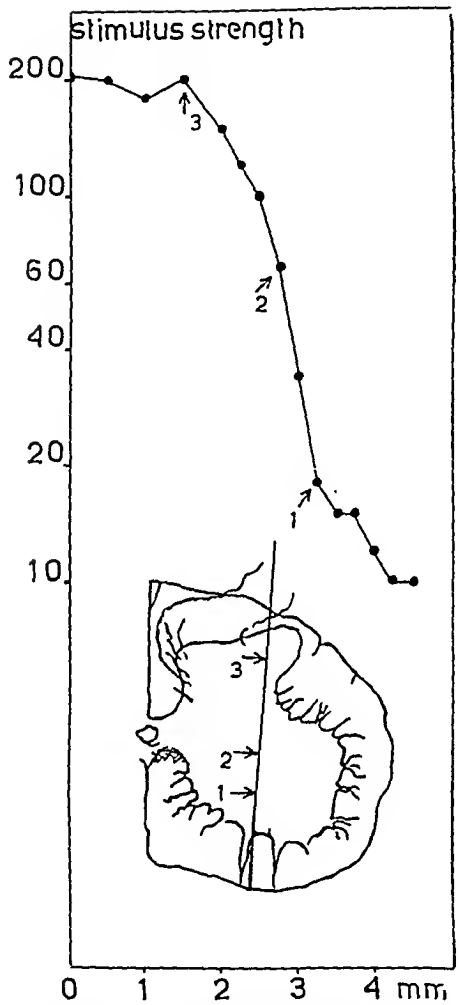

Fig. 4. Threshold curve for direct volleys in the gastrocnemius nerve.

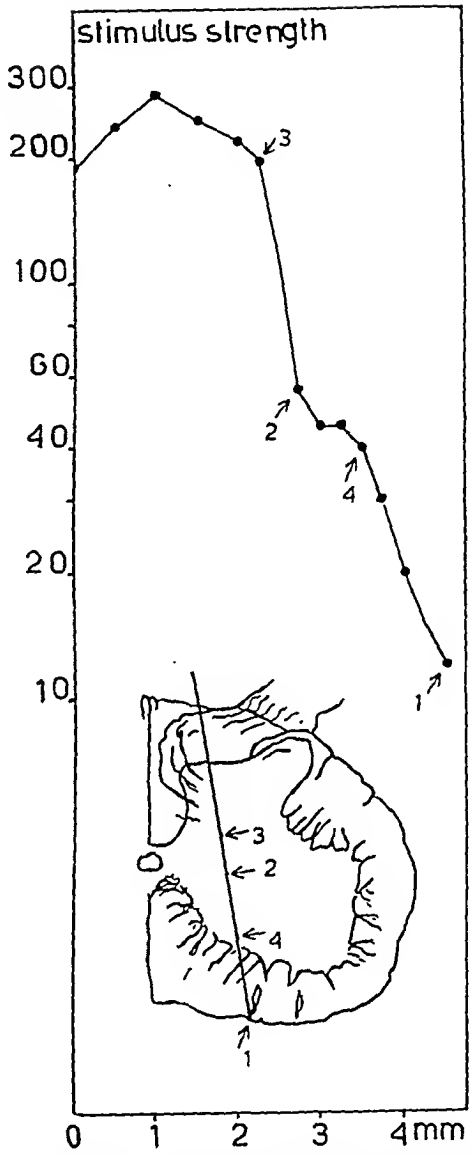

Fig. 5. Threshold curve for the gastrocnemius nerve. Same animal, side and segmental level as in Fig. 4.

rising course in its final stage and not the falling form as shown in Fig. 3. Consequently the point of the needle stimulated a bundle of axons in the area, which in the cross section is marked with arrow 1, *i. e.* those root filaments in the anterior funiculus just laterally to the end of the channel. Further, it is likely that these axons originate exclusively or mainly from cell bodies situated laterally in the anterior column, since the figure shows that the needle did not cross the axons stimulated.

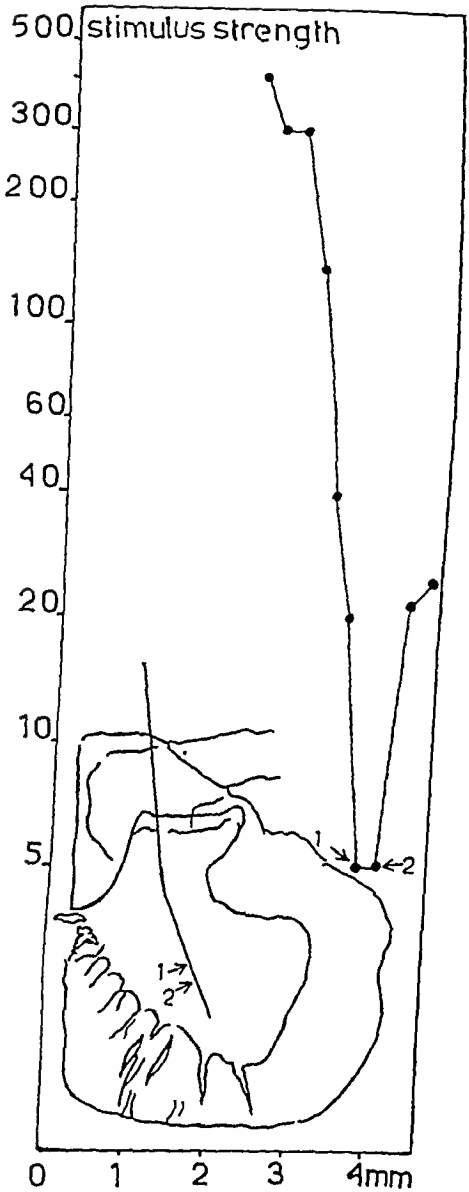

Fig. 6. Threshold curve for the gastrocnemius nerve.

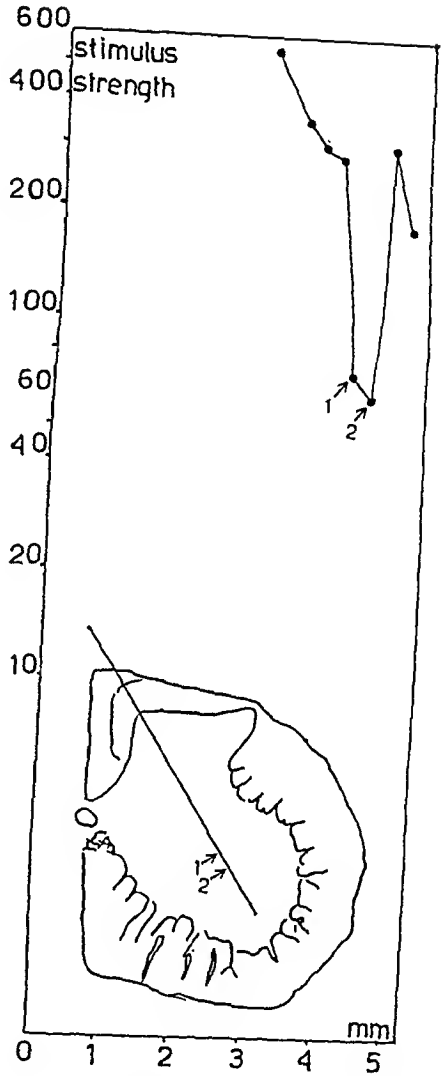

Fig. 7. Threshold curve for the gastrocnemius nerve.

In order to make their course clear the next step was to try to excite other parts of these axons. Fig. 4 illustrates an experiment, in which the direction of the needle was the same as in Fig. 3, though the electrode was placed more laterally in the anterior column. The values to the right of arrow 1 on the curve are extremely low, which indicates that dense lying elements with high excitability were stimulated. The course of the

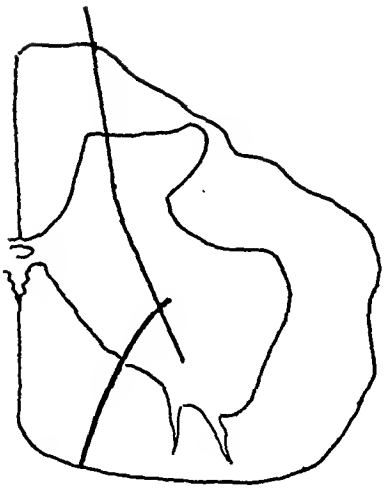

Fig. 8. Schematic picture showing the interpretation of the experiment in Fig. 6. Further description in text.

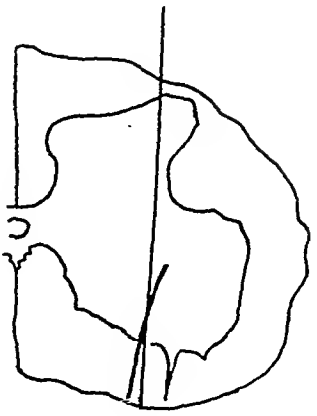

Fig. 9. Schematic picture showing the interpretation of the experiment in Fig. 4. Further description in text.

axons through the grey substance cannot be seen on the preparations made use of in this work. Fig. 4, however, gives the impression that the microelectrode crossed (arrow 1) and then passed quite close to the motor fibres which run from the lateral part of the anterior horn through the grey matter towards the ventral root filament medial to the channel within the anterior funiculus. Fig. 6 shows a curve from another experiment, which confirms this interpretation. In this case the electrode had a ventrolateral direction. The threshold curve shows a pronounced and narrow dip (marked with two arrows), indicating that the electrode crossed a narrow band of elements with great excitability *viz.* the axons in that region of the spinal cord, which is also marked with two arrows in the cross section. When comparing Fig. 4 and Fig. 6 it is obvious that in the latter experiment the electrode crossed the motor nerve fibres more or less perpendicularly, since the minimum of the curve is narrow (see Fig. 8). The broad minimum of the curve in the former experiment indicates that the electrode crossed the fibres at a narrow angle and then ran parallel with the axons (see Fig. 9). As the point of intersection between the electrode and the bundle of axons in both experiments is in the same place in the ventral column and at the same segmental level, it is peculiar that the threshold value at this point is greater in Fig. 4 than in Fig. 6. Fig. 7 shows an experiment with still higher values at the intersection. This difference in the minimum values may be due to the

electrodes passing the bundles of axons axially at different distances.

The above experiments demonstrate how it is possible to determine the intraspinal course of different parts of the motoneurons. Thus, it is shown that axons of the gastrocnemius nerve originate from lateral cell bodies. It will also be seen from the experiments (see *e. g.* Fig. 6) that these axons run in one distinct bundle towards the ventral surface and that this bundle is situated dorsomedial (*i. e.* nearer the central canal) to the axons from other cell bodies which are to be found in the lateral part of the anterior horn.

In order to determine the position of the cell pool for the gastrocnemius nerve, it is convenient to let the needle cross the axons near to their origin in the cell bodies. Fig. 10 gives an illustration from such an experiment in which the electrode during its passage through the anterior horn crossed the axons close to their origin in the motor cell bodies. The cross section shows that the minimum of the curve (arrow 1) corresponds to the most dorsal part of the lateral region of the anterior column containing motor cells. The question then arises as to whether the point of the needle in this position stimulated the cell bodies or the axons. The difficulty of actually stimulating the cell bodies locally, however, will be seen from the experiment illustrated in Fig. 11, it having been carried out on the same animal and on the same segmental level though on the other side of the spinal cord. In this case the needle penetrated the posterolateral cell group instead of passing quite close to it, as in Fig. 10. On the unstained preparations most of the cell bodies in this group actually show distinct signs of degeneration as opposed to the motor cell bodies in Fig. 10, where only some occasional cells are injured. The thresholds are high and the curve does not show any steep fall. From this and similar experiments it will be seen how difficult it is to place the point of the microelectrode sufficiently near the cell bodies to be able to excite them without injuring them. In the region marked with arrow 1 on the cross section (Fig. 10) the electrode obviously stimulated the axons coming from the cells situated just laterally of this region. Despite the fact that the end of the channel in Fig. 11 has about the same position as the point in Fig. 10 marked with arrow 1, the difference between the threshold values of these points is nevertheless considerable. Moreover, this observation shows that the current

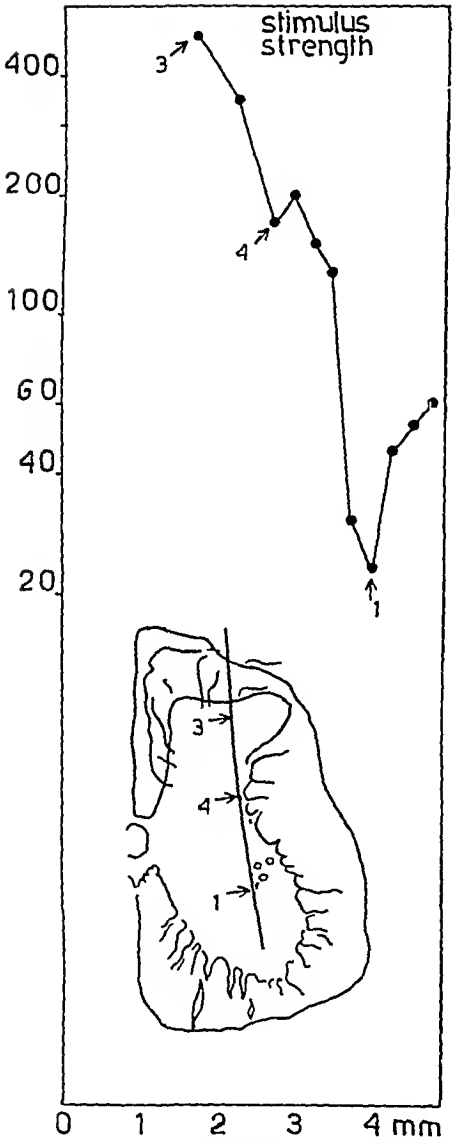

Fig. 10. Threshold curve for the gastrocnemius nerve.

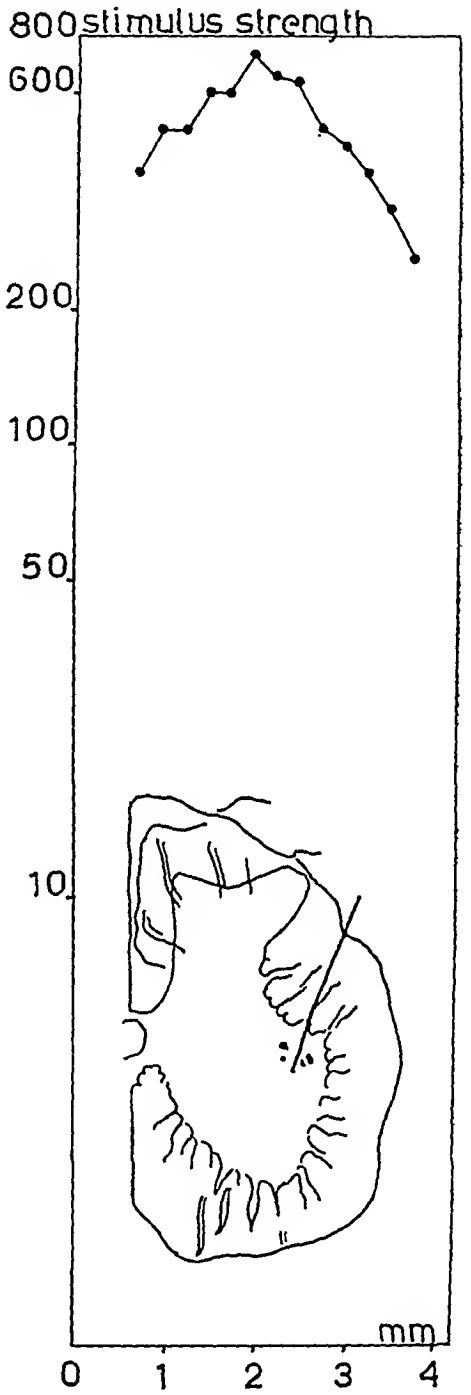

Fig. 11. Threshold curve for the gastrocnemius nerve. Same animal and segmental level as in Fig. 10 but on the other side of the cord. Black spots indicate injured cells.

spread from the needle point is small, which means that elements near the point are selectively activated. It is not even necessary to assume that the high threshold values in Fig. 11 are caused by the current spread to elements situated at some distance from the stimulated area. On the contrary, it is possible that the high values may depend on the local excitation of elements situated in the immediate vicinity of the point of the needle (*i. e.* dendrites).

Fig. 10 gives rise to further reflexions. As has already been said, the minimum of the curve corresponds to the local activation of axons. The value of this point is higher in this and also in other similar experiments than it is in the experiments in which the axons are stimulated further away from their origin (*e. g.* Figs. 3 and 6). This may be due to the fact that the axons near the cell bodies are not yet collected into one compact bundle. Further, it is remarkable that the curve falls steeply before but rises less steeply after the dip (cf. Fig. 6 where both fall and rise are equally steep). The last part of the curve (to the right of the minimum) certainly corresponds to the local activation of motor elements situated in the anterior horn ventrally of the point which is marked with arrow 1 in the cross section. It is not probable that the needle crossed nerve fibres from cell bodies in this region, for in this case the curve would have had two or more minima. According to the experiment described on page 37, the last part of the curve rather corresponds to the activation of a dense bundle of dendrites, which runs ventrally through the lateral part of the anterior column.

Fig. 10 offers a favourable position of the electrode for localizing cell groups. The needle has crossed all or nearly all the axons close to their origin in the lateral motor cell bodies. Fig. 12 shows the picture of an experiment carried out under similar conditions and it confirms the above conclusions, since the minimum of the curve corresponds to the region where the needle crossed the axons near their origin in the dorsolateral cell pool without causing any injury to the cell bodies. It should be pointed out that in this experiment the curve rises more steeply after the dip than it does in Fig. 10, which is due to the needle having taken a ventromedial direction, thus passing medially of the lateral dendrites just mentioned.

The experiments hitherto described as well as that represented in Fig. 5 have been summed up in Figs. 13 and 14. Fig. 13 illus-

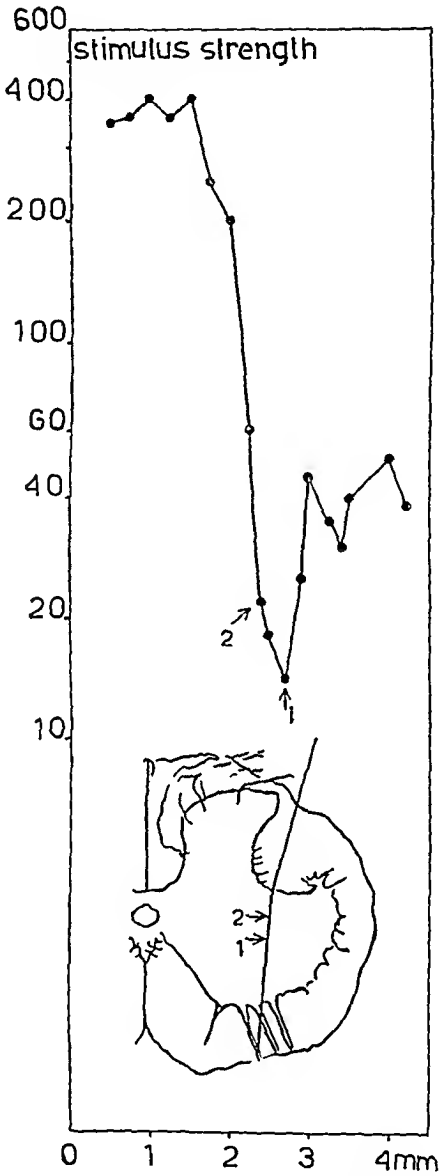

Fig. 12. Threshold curve for the gastrocnemius nerve. Upper part of the 1st. sacral segment (as against the other experiments).

trates the experiments in which the needle took a ventromedial direction and Fig. 14 illustrates those in which the needle had a ventrolateral course. The position of the cell bodies and the axons for the gastrocnemius nerve are given schematically by marking the points at which the stimulating needle struck bundles of nerve fibres according to the different diagrams. The threshold values for direct volleys when exciting these points are lower than

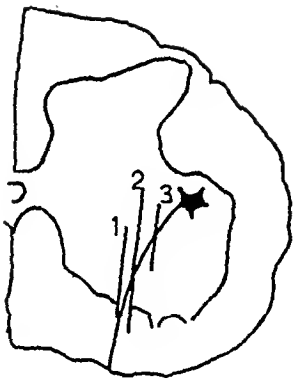

Fig. 13. Schematic picture. The position of the cell bodies and the axons for the gastrocnemius nerve are given schematically by marking the points at which the stimulating needle struck bundles of nerve fibres according to the diagrams in Fig. 3 (channel 1), 4 (channel 2) and 12 (channel 3).

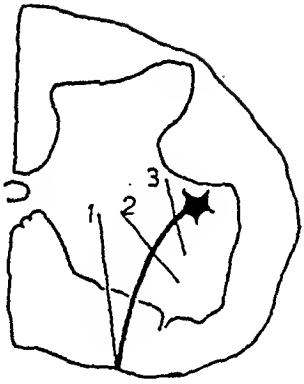

Fig. 14. Schematic picture showing the position of the cell bodies and the axons for the gastrocnemius nerve according to the diagrams in Fig. 5 (channel 1), 6 (channel 2) and 10 (channel 3).

for any other region tested. They also vary within relatively limited boundaries as compared with the threshold values of other regions. Thus, the lowest value is obtained when stimulating that part of the anterior column or anterior funiculus which contains motor axons. Special experiments have shown that the threshold values in the case of intramedullary stimulation are the same as or only slightly higher than they are when extramedullary parts of the gastrocnemius nerve fibres are stimulated, *e. g.* when the needle is placed in one of the anterior root filaments.

In the experiments now described the electrode passed through the anterior column *medial* to the lateral cell groups. As has already been pointed out, this course is the most favourable for the localization of cell bodies, since the needle crosses axons from these cells. Thus, it has been shown that the motor cell bodies for the gastrocnemius nerve are situated dorsolaterally in the anterior column. Experiments in which the electrode passed *through* this group have given varying threshold curves (see *e. g.* Fig. 11 and Fig. 20). Fig. 25 shows an experiment in which the value at the end of the curve of the gastrocnemius nerve (solid line) corresponds to a point situated just *lateral* to the dorsolateral cell bodies. This value is high and the last part of the curve does not show any steep fall. Thus, experiments with the needle in a lateral position give no information as to the position of the motor cell bodies.

b) Ipsilateral Dendrites.

Dendrites to the anterior commissure and the anterior funiculus. Direct volleys can be evoked not only from the anterior column and the anterior funiculus but also by stimulating almost any part of the segment, and it has previously been shown that such direct volleys are evoked by locally stimulating the dendrites (ÅSTRÖM 1947). From histological studies carried out on embryos and newborn animals we know that the anterior commissure contains a considerable number of dendrites all close together, belonging to the motoneurons of both sides (CAJAL 1909 *et al.*). As far as the author knows there is nothing to contradict the assumption that these commissural dendrites are to be found in adult animals too. According to the histological investigations mentioned, the dendrites pass through the anterior commissure as a dense bundle, the boundaries of which are easy to determine on the microscopic preparations. Further, this area containing only dendrites is situated at a considerable distance from other excitable parts of the motoneurons *viz.* the cell bodies and the axons.

Thus, in order to prove the method adopted for the localization of dendrites *the anterior commissure* is particularly suitable for local stimulation.

Fig. 15 shows curves where the steep fall corresponds to the passage of the microelectrode through the anterior commissure. It is particularly worthy of note that the curves within this area for the ipsi- and contralateral nerves are very similar in their general course. This does not occur when other regions of the spinal cord are stimulated, as will be seen, *e. g.* in Fig. 16, which illustrates curves from an experiment, in which the needle passed somewhat lateral to the middle line.

As the axons of the motoneurons leave the spinal cord on the side in which their cell bodies are situated (BOK 1928), the steep uniform fall of the curves at the passage through the anterior commissure in Fig. 15 can only indicate that the point of the needle excited commissural dendrites.

After the passage of the commissure the electrode (Fig. 15) continued through the most mediodorsal part of the anterior funiculus right to the anterior median fissure. When passing through the anterior funiculus the thresholds fell somewhat more, which must be attributed to the activation of the dendrites in this region. It is remarkable that the threshold values for the

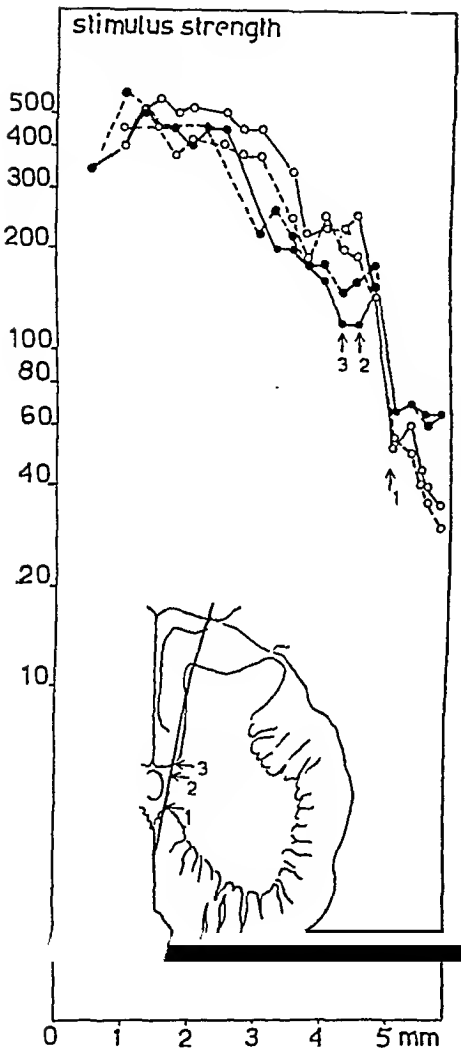

Fig. 15. Threshold curves for the gastrocnemius nerves (solid lines) and the deep peroneal nerves (broken lines) on the same side as the needle (ipsilateral nerves, filled circles) and on the other side (contralateral nerves, open circles). Same animal as in Figs. 10 and 11.

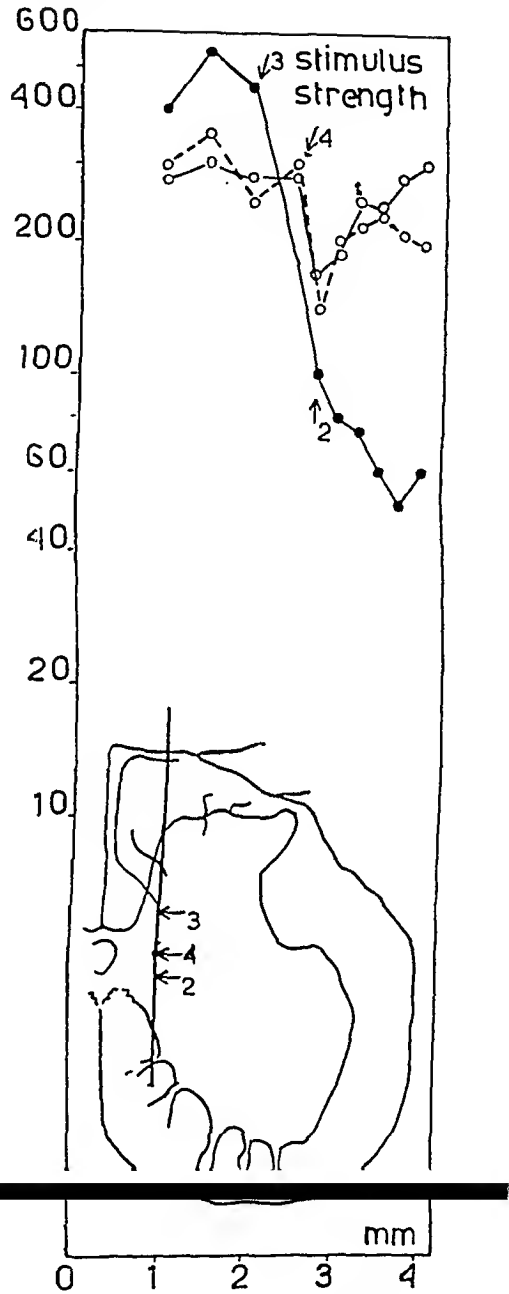

Fig. 16. Threshold curves for the ipsilateral gastrocnemius nerve (solid line, filled circles), the contralateral gastrocnemius nerve (solid line, open circles) and the contralateral deep peroneal nerve (broken line, open circles).

contralateral nerves are lower than those for the ipsilateral. This observation is confirmed in other experiments.

Fig. 16 shows that if an area lateral to the anterior commissure is stimulated, there is a considerable difference in the threshold values for the different nerves, as has already been indicated. The curve for the ipsilateral gastrocnemius nerve (solid line, filled circles) continues to fall below the point corresponding to the intersection between the electrode and the commissural dendrites (arrow 2). The curves for the contralateral nerves (open circles), on the contrary, rise again after the intersection. The following conclusions may be drawn by comparing this experiment and the one in Fig. 15. Each anterior funiculus contains dendrites from the motoneurons of both sides. The dendrites from the contralateral cell bodies are to be found especially in a narrow area quite close to the anterior median fissure, where they are even more numerous than those from the ipsilateral cells. Dendrites from cells on the same side of the spinal cord are to be found in abundance within the whole of the anterior funiculus, though perhaps they are somewhat less frequent close to the median fissure.

The curves for the contralateral nerves continue in Fig. 15 to sink further after the steep fall. This course may be interpreted in the following manner. At the point in the cross section marked with arrow 1 the needle excited a bundle of commissural dendrites, chiefly destined to the anterior column and the lateral funiculus (see below). The course of the curves to the right of this arrow corresponds to the activation of the dendrites from the other side of the spinal cord not intersected by the needle, which take a ventral turn in the anterior commissure and then run close to the anterior median fissure medial to the deepest part of the channel (cf. the schematic diagram in Fig. 40 p. 61).

Dendrites from the cell bodies on one side of the spinal cord may run to the other through the posterior commissure as well. As will be seen in Fig. 15, however, the part of the curves corresponding to this area (marked with arrow 2 and 3), does not show the similar and easily interpreted course which is characteristic of the anterior commissure. Inasmuch as dendrites are to be found within the posterior commissure, they are far less dense and occur less regularly than in the anterior commissure.

As the motor cell bodies of the gastrocnemius nerve are situated posterolaterally in the anterior column, the commissural den-

drites from these cells might be expected to run horizontally through the grey matter towards the anterior commissure. As a matter of fact this is evident, too, from curves from most of the experiments, where the microelectrode passes through the anterior horn between the cell bodies and the anterior commissure. In Fig. 16 arrow 2 indicates the spot where the curves show an increased density of excitable elements. This is particularly striking for the curves of the contralateral nerves. This point corresponds to an area on a level with the anterior commissure (see the cross section). It is clear that in this position the needle stimulated dendrites to or from the anterior commissure. Their course will now be followed to the origin:

Fig. 5 illustrates an experiment, in which the needle passed still further from the middle line than in the two already described. The threshold curve is composed of two falling and two horizontal phases. The part of the curve to the left of arrow 4 shows that, after passing an area in which the dendrites are less dense, the needle reached a region dense with dendrites. The boundary between these two regions is marked in the cross section with arrow 2. It is obvious that the point has here stimulated a bundle of dendrites, which according to the analysis previously made run to the anterior commissure.

The horizontal part of the curve (enclosed by arrows 2 and 4) shows that the electrode passed a region homogeneously and densely filled with dendrites. The cross section shows that the dendrites originating in the dorsolateral cell bodies run through the medial part of the anterior column to the part of the anterior funiculus, where a comparative abundance of dendrites has already been shown (Figs. 15 and 16).

The last part of the curve shows a falling course, which would correspond to a dense region of dendrites. It is obvious from the cross section that if this is the case, these dendrites must be situated within that part of the anterior funiculus which contains ventral root fibres. As the terminal of the channel corresponds to a bundle of ventral root fibres, it cannot be altogether excluded that this fall in the curve implies a current spread to close lying elements with high excitability, *i. e.* motor axons. If the current spread is not greater than it is in the posterolateral part of the anterior column (see p. 24), and if the diminishing threshold values really depend on current spread to the ventral root filaments, then the curve would, however, have a much steeper

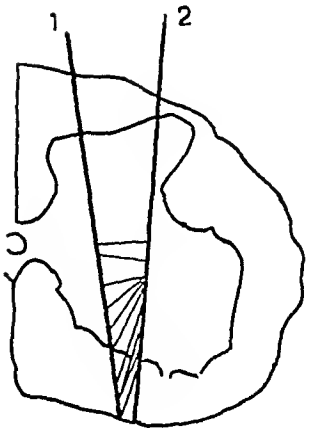

Fig. 17. Schematic picture drawn on the basis of the experiments in Fig. 4 and 5. The points along channel 1 (Fig. 5) and 2 (Fig. 4) having equally large threshold values are connected by straight lines. Further description in text.

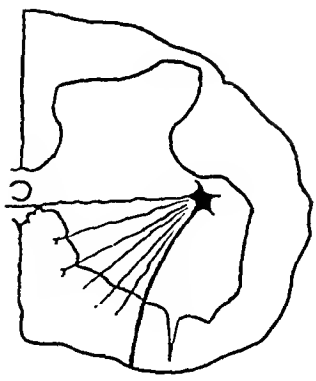

Fig. 18. Schematic diagram on the basis of Figs. 13, 14 and 17 showing the position of the medioventral system of dendrites.

fall than it actually has. Thus, the experiment illustrated in Fig. 5 indicates that a considerable number of dendrites from the dorsolateral cell pool (the gastrocnemius nerve) spread to the anterior funiculus.

Fig. 4 (see p. 21) shows an experiment in which the needle passed through the anterior column still more laterally than in Fig. 5, *i. e.* nearer the origin of the dendrites described. The curve in Fig. 4 falls evenly down to the point at arrow 1, which corresponds to the motor axons (see above). This curve does not show any hump corresponding to the commissural dendrite bundle, as can be seen from the curve in Fig. 5. This is due to the fact that the channel in Fig. 4 crosses the dendrites so near to their origin in the cell pool that they have not yet spread to different regions of the spinal cord (the posterior column, the anterior column and the anterior funiculus). The experiments in Fig. 4 and Fig. 5 are illustrated in Fig. 17, in which the points along the two channels 1 and 2 having equally large threshold values are connected by straight lines. It must be emphasized that threshold values from different experiments cannot be compared as a rule. In this case, however, direct comparison is permissible, since the experiments have been carried out on the same animal, on the same segmental level and on the same side of the spinal cord. Further, the electrode was situated

medial to the cell pool in both of the experiments, thus intersecting the same system of dendrites. The figure shows that the points with equal threshold values are more dispersed along channel 1 than along channel 2. This means that the dendrite field spreads in a fanlike fashion medially between the horizontal

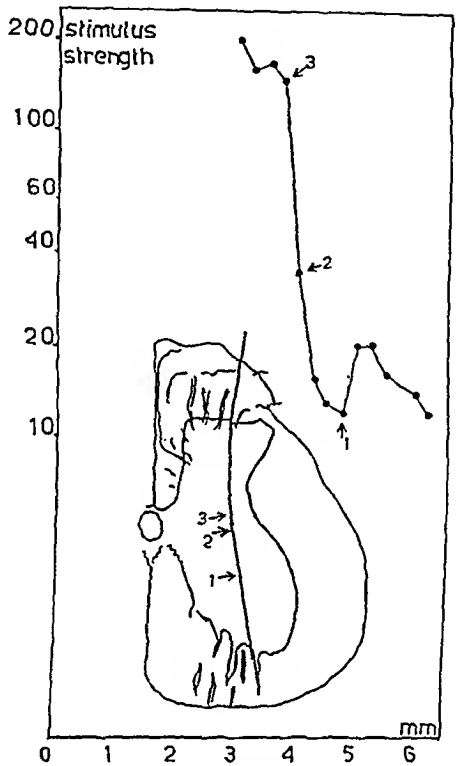

Fig. 19. Threshold curve for the gastrocnemius nerve.

bundle of dendrites and the motor nerve fibres directed towards the ventral surface. On the basis of these experiments and the knowledge concerning the position of the cell bodies and axons (Figs. 13 and 14), the schematic diagram has been drawn in Fig. 18. It will be seen from this figure that the needle intersects the medial system of dendrites near the cell pool across a shorter stretch than it does further away from the cell bodies (as in Fig. 5). Fig. 12 (p. 27) illustrates an experiment in which the electrode passed quite close and medial to the cell pool of the gastrocnemius nerve. There is a pronounced dip in the curve at arrow 1, and this dip corresponds to the activation of an area medial to the posterolateral cell group. It has already been shown that the lowest point of the curve (arrow 1) represents activation of

motor axons. The steep fall before this minimum, however, stops at a value corresponding to a point (arrow 2) which is situated about 0.4 mm dorsal to the point at which the motor axons are activated. It is obvious that the needle between arrow 2 and arrow 1 in the cross section intersects the majority of the dendrites, the branches of which subsequently spread in a medial direction to the anterior horn and the funiculus. Fig. 19 illustrates an experiment carried out under similar conditions. The distribution of the threshold values shows a great similarity to that in the experiment just described. The value at arrow 1 indicates the activation of the axons running ventrally through point 1 in the cross section, and the value at arrow 2 represents — as in Fig. 12 — the dorsal boundary for the medial system of dendrites just mentioned.

The above results may be summarized in the following manner (*cf.* also the schematic picture in Fig. 40, p. 61). From the medial side of the cell pool of the gastronemius nerve there emerge, firstly axons collected in a bundle running towards the ventral surface of the cord, secondly an abundant collection of dendrites having a medial and anteromedial course. They spread out in a fan-shaped fashion over the anterior column and the anterior funiculus of the same side. Further, a bundle of dendrites runs through the anterior and possibly also the posterior commissure to the grey and white matter of the other side, the distribution being described on page 44.

The stimulation of the *lateral and anterolateral parts of the anterior column* gives threshold curves more irregular and difficult to interpret than those representing the region medial to the cell pool of the gastrocnemius nerve. This may be due, partly to the cell bodies of the motoneurons being destroyed in varying degrees, and partly to technical difficulties in connection with the selective activation of dendrites without simultaneously stimulating the adjacent axons in this region. The results of the experiments in this region must consequently be interpreted with greater reserve than previously. Fig. 20 shows an experiment in which the electrode passed near the lateral border of the anterior column and then continued into the anterior part of the lateral funiculus. The steep fall of the curve for the gastrocnemius nerve begins at a point (arrow 1) corresponding to the posterolateral part of the anterior

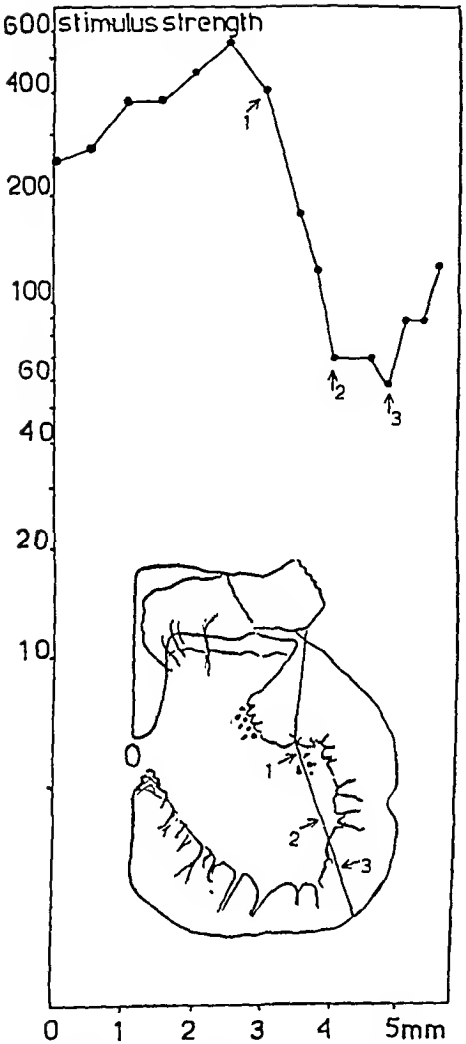

Fig. 20. Threshold curve for the gastrocnemius nerve. Black spots indicate injured cells.

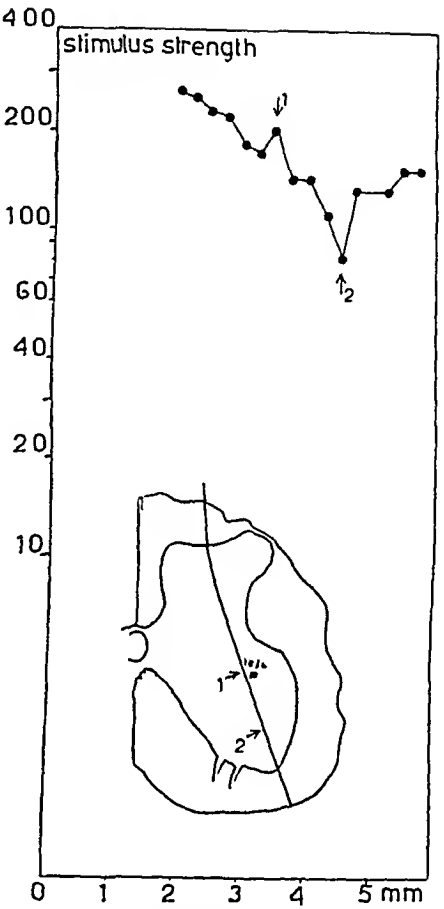

Fig. 21. Threshold curve for the gastrocnemius nerve.

horn. The point of the needle passed through or close to the cell pool of the gastrocnemius nerve and the threshold values of the corresponding part of the curve are high (cf. page 24). From the point at arrow 1 the curve falls steeply to a broad minimum, representing an area at the border between the grey and white matter (between arrows 2 and 3). Obviously the electrode has excited a dense collection of dendrites. These dendritic arborizations may come either from the posterolateral cell bodies or from the main branches of the dendrites, which in the lateral part of the anterior column lie medial to the channel (se below).

Fig. 21 represents an experiment, in which the electrode is also directed towards the lateral funiculus, though it has penetrated the anterior column more medially and ventrally than in the experiment just described. There is no minimum in the threshold curve corresponding to the area close to the cell pool of the gastrocnemius nerve (marked with arrow 1 in the cross section). This must certainly be attributed to injury caused by the microelectrode, for in the microscopical sections bleeding and distinct degeneration can be observed in the cell bodies situated in the vicinity of the channel. The threshold curve for the gastrocnemius nerve then falls and shows comparatively low values for the rest of its course, corresponding to the anterolateral part of the anterior column and the lateral funiculus. There is a dip in the curve at arrow 2 which may correspond to the activation of motor axons situated at point 2 (in the cross section) or else motor cell bodies just lateral to it. The following reasons, however, contradict such an assumption: 1) The experiments in Fig. 10 (p. 25) and Fig. 12 (p. 27) showed clearly that the cell bodies for the gastrocnemius nerve are to be found posterolaterally and probably only there. 2) The experiments illustrated in Figs. 13 and 14 showed that the curve obtained by activating motor axons is characterized by low threshold values at the point where the needle passed the axons. In Fig. 21 the dip, however, is wide and shallow, which rather indicates a stimulation of dendrites extending over a certain area around the point at arrow 2.

Thus, it is obvious that the part of the curve to the right of arrow 1 in Fig. 21 corresponds to the activation of dendrites extending over the lateral part of the anterior column and the lateral funiculus. The course of the curve indicates that the dendrites are particularly dense around the point marked with arrow 2 in the cross section. Fig. 37 (p. 55) represents another experiment in which the needle has passed through the lateral part of the anterior column. Arrow 4 marks at the curve of the gastrocnemius nerve (solid line) the value which corresponds to the stimulation of the dendrites just described.

Fig. 22 illustrates an experiment, in which the needle has passed through the lateral part of the anterior column. The part of the curve (to the left of arrow 1), which corresponds to the posterolateral part of the anterior horn (situated dorsally of arrow 1 in the cross section) shows high values and no steep fall. The dip of the curve to the right of arrow 2 corresponds to the anterior part

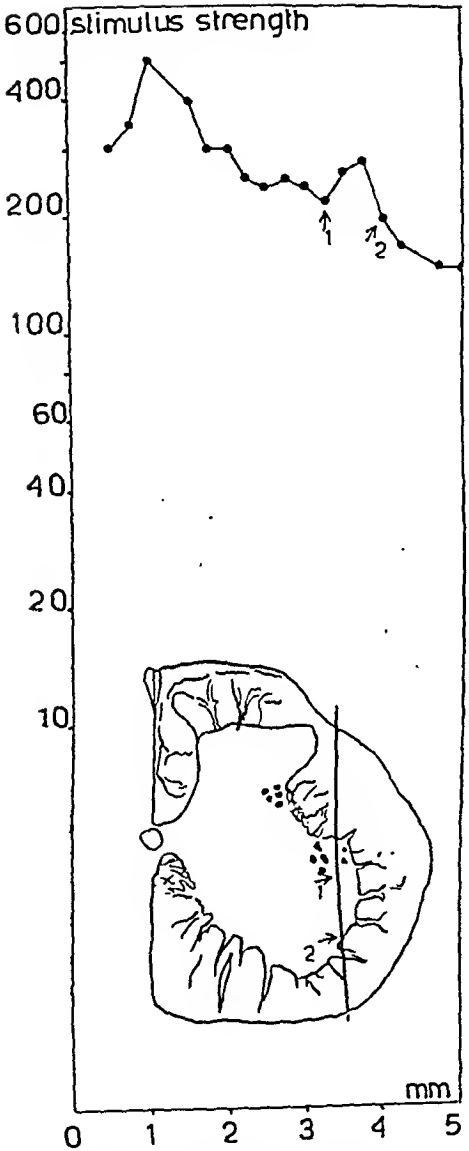

Fig. 22. Threshold curve for the gastrocnemius nerve. Black spots indicate injured cells.

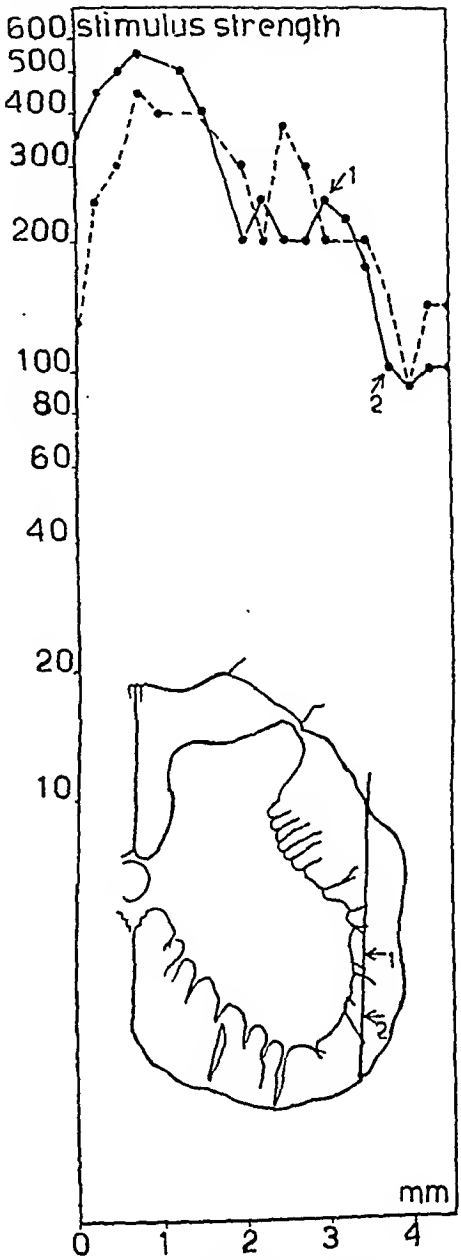

Fig. 23. Threshold curves for the gastrocnemius nerve (solid line) and the deep peroneal nerve (broken line).

of the lateral funiculus, thus showing that this region contains a fairly dense collection of dendrites. In Fig. 22 the channel has a more lateral position than in Fig. 21, consequently it does not cross the dense bundle of dendrites running around point 2 in the cross section in Fig. 21. Fig. 23 represents an experiment, in which

the needle penetrated the lateral funiculus. The slow fall of the curve of the gastrocnemius nerve (solid line) beginning at arrow 1 represents an activation of dendrites, the density of which increases ventrally. The minimum of the curve to the right of arrow 2 shows that a considerable number of elements ventral to point 2 in the cross section are stimulated, which indicates that dendrites to the anterior part of the lateral funiculus have been stimulated.

Hence, all the experiments now described show that a dense bundle of dendrites emerges from the ventral side of the cell pool of the gastrocnemius nerve and runs ventrally towards the anterior part of the lateral funiculus, there giving off a number of terminal arborizations. The dendrites also send out branches to the lateral part of the anterior column and to the part of the lateral funiculus situated lateral to the ventral horn.

Direct volleys can be evoked from considerable parts of the *posterior column*. The threshold values for these waves are higher than they are when stimulating the region so far dealt with, *i. e.* the ventral part of the spinal cord. Fig. 19 shows an experiment in which the needle passed through the posterior and anterior columns. It has already been shown that the value at arrow 2 in the curve corresponds to the activation of the bundle of dendrites running towards the anterior commissure. To the left of arrow 3 the curve does not show any steep fall, as is the case between 3 and 2. This means that this part of the curve (left of arrow 3) is not due to the spread of the stimulating current to motor elements in the anterior column, but represents a local activation of dendrites in the posterior column dorsal to point 3 in the cross section. Fig. 3 illustrates a similar experiment, which still more clearly shows that the threshold values to the left of arrow 3 represent the local stimulation of dendrites in the posterior column dorsal to point 3 in the cross section. This point is just about as deep in the posterior column as the corresponding point in Fig. 19.

Fig. 5 illustrates an experiment in which the needle passed medially through the posterior column. The figure shows that the curve, as in Fig. 19, has a slight slope with high values to the left of arrow 3, which represents an area in the posterior column dorsal to point 3 in the cross section, and subsequently a steep fall to the point (arrow 2), which corresponds to the excitation of the commissural dendrite bundle (see Fig. 17 above, p. 33). Fig.

16 shows a similar course of the ipsilateral gastrocnemius nerve (filled circles, solid line) in the curve when the needle passed still further medially through the posterior horn. The curve begins to fall at a point (arrow 3) corresponding to the deep part of the posterior column. The even fall ceases at the point (arrow 2), which corresponds to the activation of the commissural dendrite

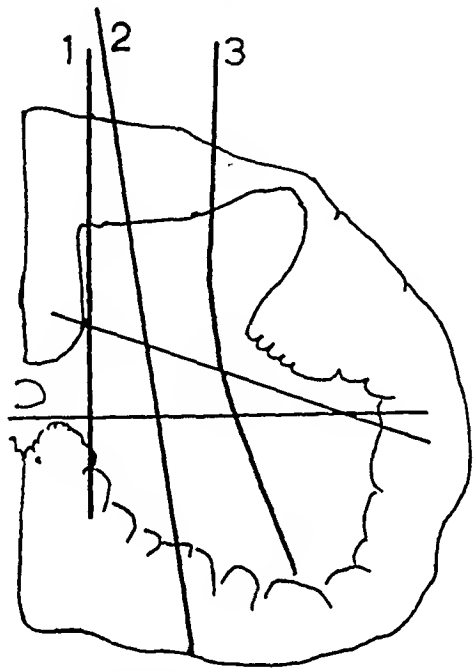

Fig. 24. Schematic diagram on the basis of the experiments in Fig. 16 (channel 1), 5 (channel 2) and 19 (channel 3). Further description in text.

bundle. Thus, the threshold curves for the experiments now dealt with are remarkably similar within those parts that correspond to the dorsal part of the cord. They are composed of two parts, the first with high, uniform values indicating that there are dendrites of sparse density homogeneously spread over a certain area in the posterior column, the second with a steep fall ending at a point (arrow 2) corresponding to the local excitation of the horizontally running bundle of dendrites directed towards the anterior commissure. It must not be altogether excluded that the fall of the curve is due to the current spread to this bundle of dendrites. The following, however, will show that this is not probable.

The three experiments are summarized in Fig. 24. The position of the needle channels and points 3 and 2 on the cross sections are indicated in the same picture, and lines have been drawn

through the points with the same numbers. The line through point 3 indicates the ventral boundary for the area with sparse dendrites in the posterior column. The line through point 2 indicates the dorsal boundary for the dendrites to the anterior commissure. These lines intersect each other in the dorsolateral part of the anterior column. The fall in the curve between arrows 3 and 2 corresponds to the wedge-shaped area situated between these lines. The dendrites destined to the anterior commissure are, according to analyses already carried out, collected in a homogeneous bundle. If the falling part of the curves were caused by the current spread to this bundle, the fall would correspond to an equal number of units (mm) on the horizontal axes in the three experiments. This is not the case. Thus, it is likely that the wedge-shaped area between the lines mentioned contains a system of dendrites, which can be excited locally and selectively. Fig. 24 shows that they are destined to the deepest part of the posterior funiculus and to the area just lateral to the central canal. Some of these dendrites may run through the posterior commissure (see above p. 31). Fig. 24 gives the impression that these dendrites form a special system, which, with their origin in the posterolateral cell pool and with a medial direction, spread across the wedge-shaped area indicated by the two lines. It must not, however, be excluded that they may consist of short branches from the commissural dendrite bundle spreading dorsally over the area mentioned.

In the experiments now described all the curves have had a similar course. In the case of stimulation in the lateral part of the posterior column the curves assume another shape. Fig. 4 illustrates such an experiment. The curve begins to fall at arrow 3, the cross section showing that this value corresponds to a point (3) situated dorsally in the posterior column. Arrow 2 indicates the approximate position of the value corresponding to the stimulation of the commissural dendrite bundle (see above Fig. 17 p. 33). The part of the curve between arrows 3 and 2 falls evenly. It demonstrates the existence of dendrites with an increasing density. From the cross section it is clear that these dendrites are situated in the lateral part of the posterior column dorsal to the commissural dendrite bundle. This system cannot be found in the medial part of the posterior column, which will be seen when comparing Figs. 4 and 5. These two experiments were carried out on the same animal. Arrow 3 in both figures indicates the value at which the curves begin to fall. In Fig. 5 it corresponds to the ex-

citation of a point ventral in the posterior column as opposed to Fig. 4 in which it represents the stimulation of a point dorsal in the lateral part of the posterior column. In Fig. 5 the falling part between arrows 3 and 2 represents, as already described, the excitation of a limited bundle of dendrites running to the posterior commissure. It is obvious that the electrode has excited another system in this region apart from the one corresponding to the less steep fall between arrows 3 and 2 in Fig. 4. Hence, the values in these parts of the curves cannot therefore be directly compared. On the contrary, a comparison was possible between the values corresponding to the activation of the anterior column in these experiments, since the electrode in this region intersected the same system of dendrites (see Fig. 17, p. 33). The threshold values marked with arrow 3 in both the experiments can only be compared inasmuch as they indicate the ventral boundary for the area already described in the posterior column which contains dendrites of less density.

The system of dendrites in the lateral part of the posterior column now described could not be shown in Fig. 19, consequently it must be situated within that part of the posterior horn lateral to the needle channel in this experiment. It is obvious that a separate system of dendrites from the posterolateral cell pool runs dorsally, forking with decreasing density over a fairly narrow area in the most lateral part of the posterior column.

Fig. 10 represents an experiment, in which the needle took a still more lateral direction. As in Fig. 4 the curve shows a slow fall from a value corresponding to a point dorsally in the posterior horn (arrow 3). The curve has a little notch at arrow 4, which corresponds to a point in the spinal cord where the needle was quite close to the lateral funiculus. The electrode may have excited a bundle of marginal dendrites running along the border between the posterior column and lateral funiculus (cf. CAJAL 1909).

The curves in Fig. 23 illustrate the existence of dendrites in the *lateral funiculus*. It has already been pointed out (see page 39) that they are most dense in the ventral part, and that these ventral dendrites form terminal arborizations of the collection, which runs through the lateral part of the anterior column. Fig. 25 shows an experiment in which the needle penetrated the dorsal part of the lateral funiculus. The slow fall in the curves of the

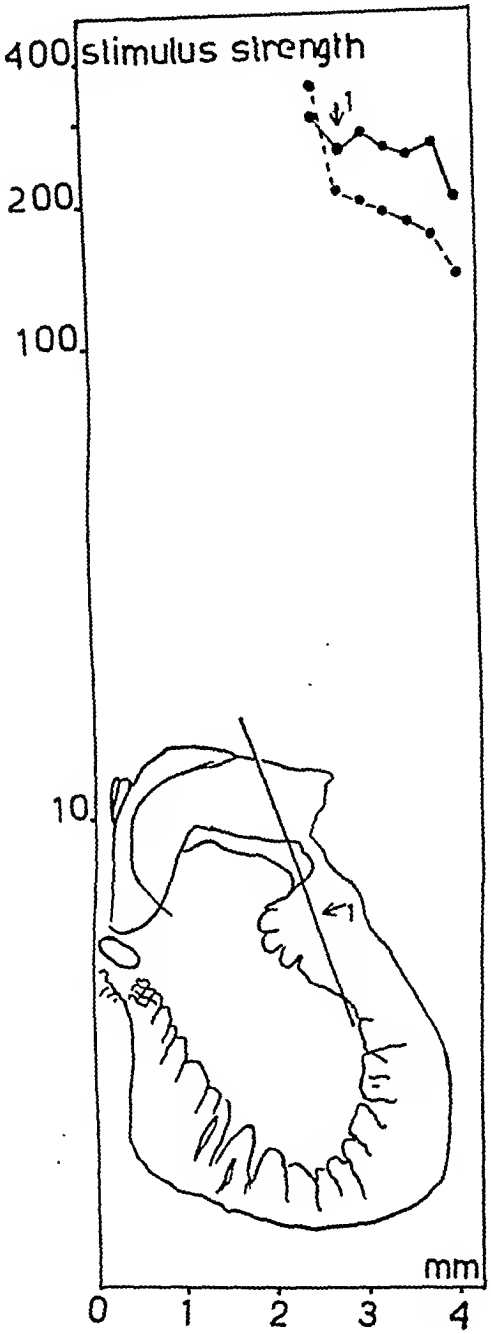

Fig. 25. Threshold curves for the gastrocnemius nerve (solid line) and the deep peroneal nerve (broken line).

two nerves (right of arrow 1) shows that there are dendrites in that part of the lateral funiculus which is lateral to the cervix of the posterior column (ventral to point 1 in the cross section).

These may originate either from the cell bodies direct or from the system of dendrites in the lateral part of the posterior column already described.

C. Contralateral Dendrites.

On page 29 it was shown that a dense bundle of dendrites runs through the anterior commissure to the contralateral side of the spinal cord. A considerable number of these run to the medial part of the anterior funiculus (see above). The following will show how the rest of the dendrites takes a lateral direction, extending over a considerable part of the grey matter.

Fig. 16, (p. 30) represents an experiment, in which the electrode passed through the grey matter about 0.5 mm lateral to the anterior commissure. The curves for the contralateral nerves (open circles) have a narrow dip (arrow 2), corresponding to a point lateral to the anterior commissure. It is obvious that the needle has excited a rather distinct bundle of dendrites, which runs laterally *from* the anterior commissure. They are to be found in the same place as the bundle of ipsilateral dendrites that is directed *towards* the anterior commissure (see p. 32).

Fig. 15 (p. 30) shows that the commissural dendrites belonging to the nerves of both sides are just about equally developed. In Fig. 16, however, the value (arrow 2), corresponding to the activation of these dendrites at some distance from the commissure, is lower in the ipsilateral nerve than in the contralateral ones; this observation shows that the bundle of dendrites belonging to the ipsilateral nerve is more developed than the one belonging to the contralateral nerves. Other experiments have shown similar results. This asymmetry in the distribution may be explained by either or both of the following reasons: 1) The contralateral commissural bundle may have given off branches within an area situated between the anterior commissure and the channel, and is consequently less in point 2 (the cross section in Fig. 16) than in the anterior commissure. 2) Genuine asymmetry may exist in this experiment, *i. e.* the contralateral dendrites belonging to the cell bodies of one side are more strongly developed than those of the other. It has not been possible to show any such asymmetry, however, in experiments where the needle has intersected the anterior commissure (see Fig. 15).

Further reflexions may be put forward in consequence of the curves of the contralateral nerves in Fig. 16. The first part (to

the left of arrow 4) is almost horizontal, which proves that there is no spread of current from the posterior column to the commissural dendrites just described (note that the curve of the deep peroneal nerve even rises before it begins its steep fall at arrow 4). Moreover, it is worthy of note that the curves for the contralateral nerves are here lower than the one for the ipsilateral gastrocnemius nerve. This shows that the spread of current cannot have taken place to the other side either. Hence, this part of the curves of the contralateral nerves (left of arrow 4) corresponds to the local stimulation of a sparse collection of dendrites spread over the medial part of the posterior column (dorsal to point 4 in the cross section). These dendrites might be expected to form a series of branches from the anterior commissural bundle. The curves of the contralateral nerves make a steep fall between arrows 4 and 2, which corresponds to a narrow area between the scanty dendrites of the posterior horn (ventral boundary point 4 in the cross section) and the commissural dendrite bundle (point 2). It is obvious that this area marks a sharp boundary between the two systems of dendrites. The experiment does not support the assumption that branches from the anterior commissural dendrites run dorsally and spread across the medial part of the posterior column, for in such a case the curves would have had an even and not a particularly steep fall as in Fig. 4 (left of arrow 2), where the electrode passed through the lateral part of the posterior column containing posterolateral dendrites. Fig. 15 illustrates an experiment in which the electrode passed just lateral to the central canal. From the value at arrow 2, which corresponds to the posterior commissure (point 2 in the cross section), the curves of the contralateral nerves fall steeply to the value at arrow 1, this figure corresponding to that part of the anterior commissure containing the principal part of the commissural dendrite bundle. As in the previous experiment, the steep fall does not indicate that dendrites run from the anterior commissure dorsally, parallel with and around the needle channel. Thus, the experiments in Fig. 15 and Fig. 16 do not indicate that the dendrites to the medial part of the posterior column emanate from the dendrites of the contralateral bundle that pass through the anterior commissure, but it is more likely that they originate from the contralateral dendrites running through the posterior commissure. Other experiments indicate that this commissure contains dendrites (see page 31 and 41).

Thus, it has been shown that dendrites from one side of the cord

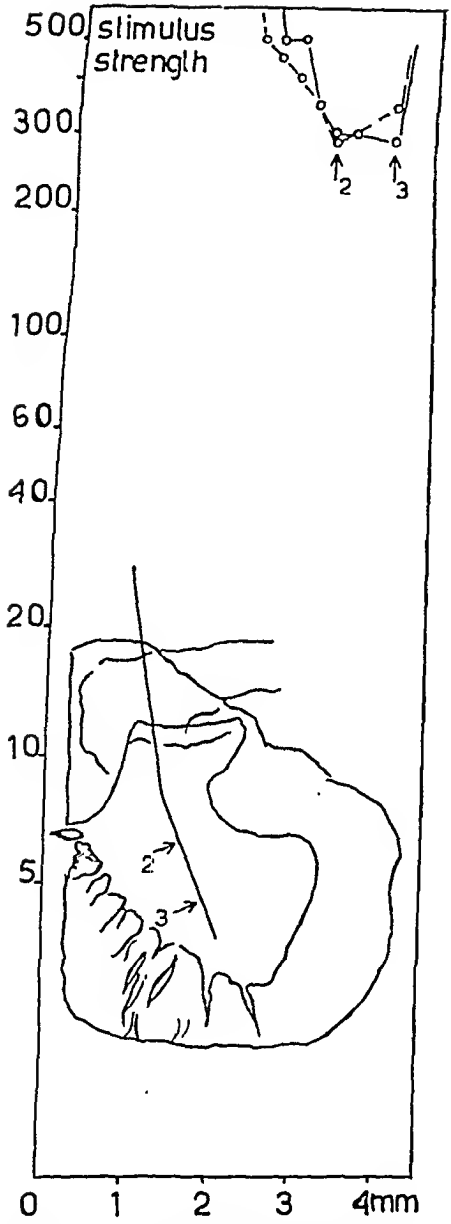

Fig. 26. Threshold curves for the contralateral gastrocnemius nerve (solid line) and deep peroneal nerve (broken line). Same experiment as in Fig. 6.

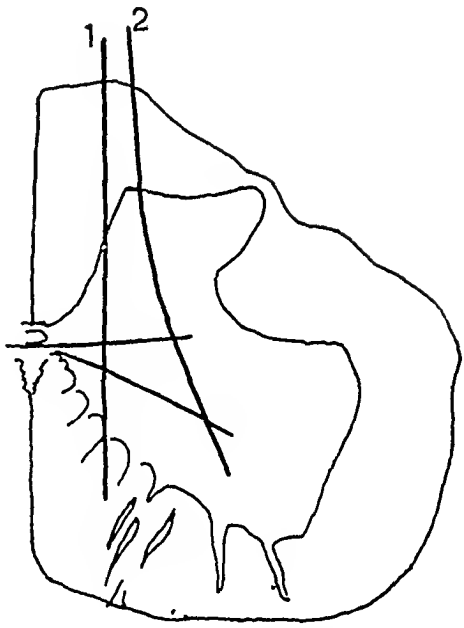

Fig. 27. Schematic picture drawn on the basis of the experiments in Fig. 16 (channel 1) and Fig. 26 (channel 2). Further description in text.

run to the other through the anterior commissure and possibly also through the posterior. The next step in the investigation will be to examine the continued course of the anterior commissural dendrites.

Fig. 26 illustrates an experiment in which the electrode passed

more laterally through the anterior column. Since the threshold values are high, it must be excluded that they are due to current spread to the contralateral dendrites in the medial part of the grey matter (their existence was shown in connection with Fig. 16). That such is not the case is evident from the comparison made between this curve and those from other experiments, where the needle passed still more laterally in the spinal cord. It will be seen that the threshold values are not higher when the needle is more lateral (as in Fig. 29 or Fig. 31) than when it is in a more medial position (as in Fig. 26). Thus, it is obvious that these threshold curves represent local activation of contralateral dendrites spreading from the anterior commissure in a lateral direction over the grey matter.

The curve for the contralateral gastrocnemius nerve, (continuous line) in Fig. 26 has a broad minimum between the values at arrows 2 and 3. This shows the existence of dendrites spreading between points 2 and 3 in the cross section, *i. e.* within the posterior part of the anterior column. It is clear that the anterior commissural dendrites, which are collected near the anterior commissure in a distinct bundle (point 2 Fig. 16) spread out more laterally over a large area (between points 2 and 3 in Fig. 26). Fig. 27 represents a schematic picture of this interpretation.

The curve in Fig. 26 falls less steeply to the value at arrow 2, which may indicate that the dendrites just described (between points 2 and 3) give off branches in a dorsal direction across the ventral part of the dorsal column. Notice the difference between this course and the steep fall (between arrows 4 and 2) in Fig. 16, which marks the boundary between two different systems of dendrites.

Fig. 28 illustrates an experiment where the needle took a ventromedial direction. The curves have a minimum (left of the value at arrow 3), which shows the existence of dendrites dorsal to point 3 in the cross section. It is evident that these dendrites in the posterior part of the anterior column correspond to those described in connection with Fig. 26. The curves then rise to the values at arrow 4 and afterwards fall again, which fall indicates the existence of dendrites situated ventral to point 4 in the cross section, in the vicinity of the boundary of the anterior column. These dendrites may belong to a system running from the anterior commissure and situated in the periphery of the anterior column at the boundary between this and the anterior funiculus.

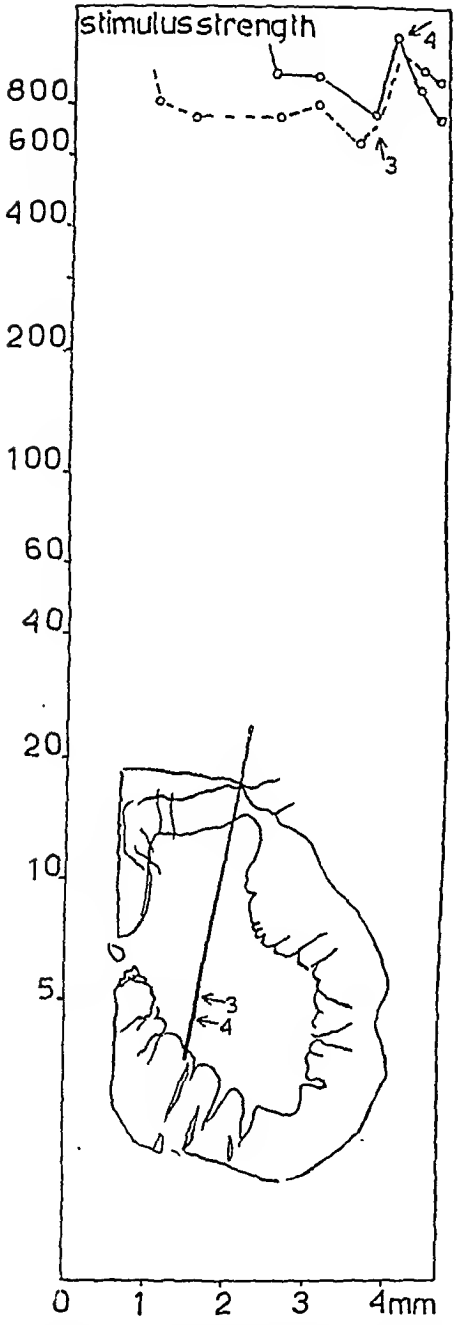

Fig. 28. Threshold curves for the contralateral gastrocnemius nerve (solid line) and deep peroneal nerve (broken line). Same experiment as in Fig. 3.

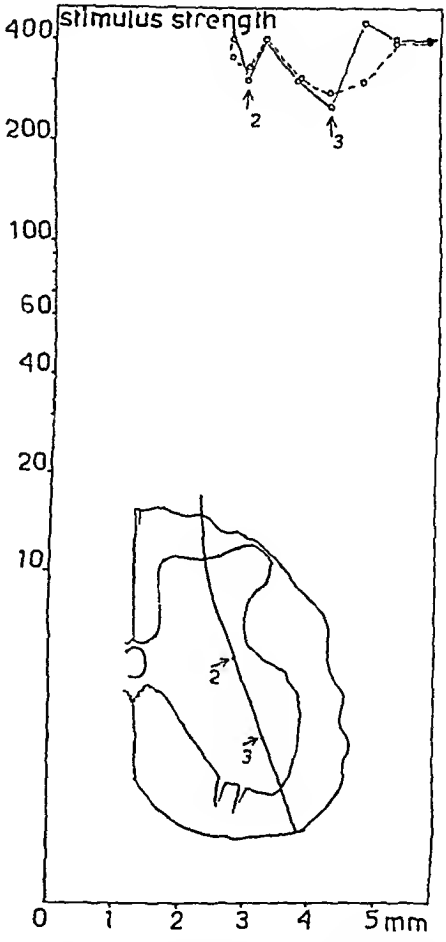

Fig. 29. Threshold curves for the contralateral gastrocnemius nerve (solid line) and deep peroneal nerve (broken line). Same experiment as in Fig. 21.

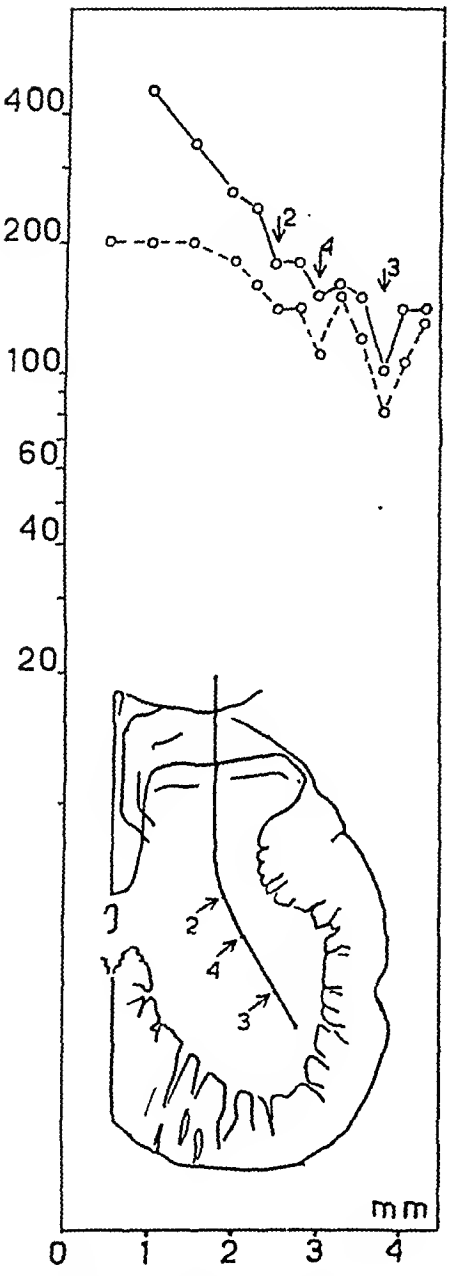

Fig. 30. Threshold curves for the contralateral gastrocnemius nerve (solid line) and deep peroneal nerve (broken line).

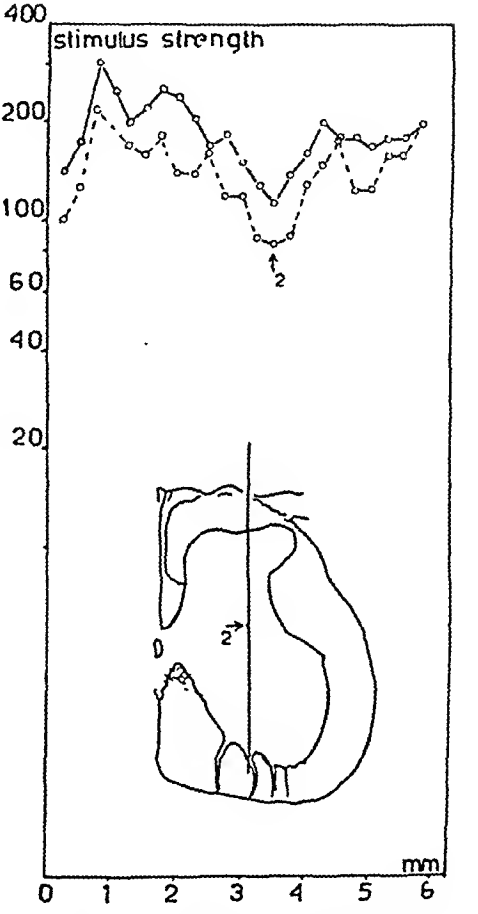

Fig. 31. Threshold curves for the contralateral gastroenemius nerve (solid line) and deep peroneal nerve (broken line).

Fig. 29 represents an experiment in which the electrode passed still more laterally through the grey matter. Once again the curves show the existence of contralateral dendrites situated in the ventral part of the posterior column and in the anterior column.

It is clear that these form the lateral continuation of those described in the two preceding experiments. As opposed to these experiments the curves have two dips, however, one narrow (lowest value at arrow 2) and one broader (lowest value at arrow 3), corresponding to an increased density of dendrites within the area round points 2 and 3 in the cross section. This and the other previous experiments imply that the contralateral dendrites running through the anterior commissure spread out across the grey matter fan-fashioned, and at the extreme lateral point they can be distinguished in two groups, one broad, situated within the anterior column with the highest density at point 3 and the other, situated more dorsally (point 2 in the cross section, Fig. 29).

The distribution of the dendrites now described has been found in other similar experiments, though there may be certain variations. Fig. 30 illustrates an experiment in which the needle had a position similar to that in Fig. 29. Arrows 2 and 3 mark the parts of the curves corresponding to the two systems of dendrites just described. The dorsal bundle (running through point 2) is less pronounced than in Fig. 29. The ventral one (through point 3) is narrower and more distinct. A third bundle running through point 4 in the cross section can be seen between these two. Fig. 31 represents an experiment in which the curves show that the dorsal group of dendrites is largest. The curve has a pronounced and broad minimum (the lowest value being at arrow 2). This value corresponds to an area in the middle of the grey matter (at point 2 in the cross section). Hence, this and the previous experiments show that there is a fairly distinct bundle from the anterior commissure running laterally across the grey matter towards the middle of the lateral funiculus. This bundle of dendrites corresponds to the one shown in Fig. 29 and can be shown in most experiments where the needle passed laterally through the grey matter.

Fig. 32 illustrates an experiment in which the needle penetrated the lateral funiculus. The curves have two dips, the one being slight at arrow 2, and the other deep at arrow 3, which, as in the previous experiments, may correspond to the activation of dendrites belonging to the two groups described.

The course of the two main collections of the dendrites is shown in the schematic picture in Fig. 33, where the position of the electrode channels in four experiments of the kind above described as well as points 2 and 3 are indicated in the same cross section.

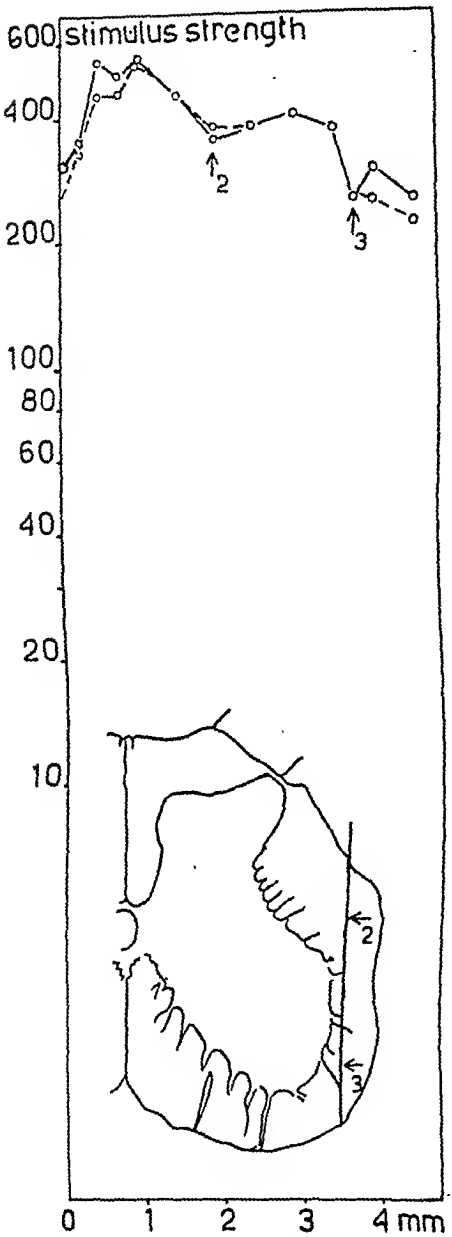

Fig. 32. Threshold curves for the contralateral gastrocnemius nerve (solid line) and deep peroneal nerve (broken line). Same experiment as in Fig. 23.

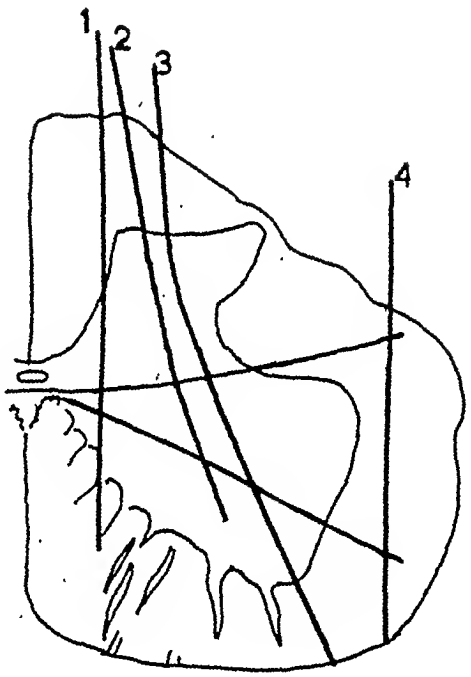

Fig. 33. Schematic picture drawn on the basis of the experiments in Fig. 16 (channel 1), 26 (channel 2), 29 (channel 3) and 32 (channel 4). Further description in text.

Thus, the two lines uniting the points 2 and 3 represent the main collections of the dendrites. It must be pointed out that they are not made up of any distinct bundles. As the previous analysis has already shown, the dendrites are spread over a large surface

of the posterior and anterior columns, and here they are more dense within the areas, whose position is marked by the two lines.

The course of the contralateral dendrites may be summarized as follows: Dendrites originating in the cell bodies of one side run to the other mainly through the anterior commissure in the shape of a dense bundle, which continues in a lateral direction. This is well collected up near the commissure but spreads further laterally over a considerable area of the grey matter, and continues out into the lateral funiculus. The dendrites are sparse in this area. Two main collections can be distinguished (see Fig. 33). One, which is usually faint, runs lateral and laterodorsal to that part of the lateral funiculus just dorsal to the dorsal boundary line for the anterior column. The other main collection, which is usually more distinct, is directed lateroventrally, ending up in the ventral part of the lateral funiculus.

It has already been pointed out (p. 31) that a fairly dense bundle of dendrites takes a ventral turn in the anterior commissure and then runs to the medial part of the anterior funiculus.

Finally the experiments indicate that a small bundle runs through the posterior commissure to the medial part of the posterior column.

Local Activation of the Intraspinal Parts of the Motoneurons Belonging to the Deep Peroneal Nerve.

When activating the cranial part of the seventh lumbar segment of the spinal cord, the threshold values of the deep peroneal nerve are lower than they are in the case of the gastrocnemius nerve, and when the caudal part of the same segment is activated, it is the reverse. This experimental finding must be referred to the fact that the cell column of the deep peroneal nerve is situated more cranial than that of the gastrocnemius nerve (SHERRINGTON 1892).

This work, however, will only deal with the localization of the motoneurons in the transversal plane. Consequently the experiments have usually been carried out by activation of the middle part of the L7 segment, the motoneurons belonging to both nerves here being considerable in number. In the different experiments there are variations which are certainly due to post- or prefixation (SHERRINGTON 1892).

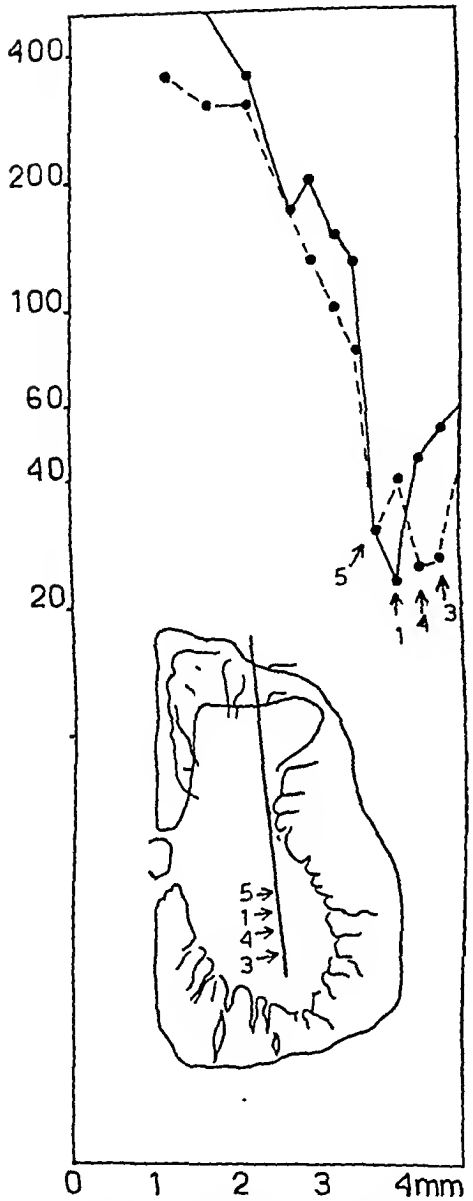

Fig. 34. Threshold curves for the gastrocnemius nerve (solid line) and the deep peroneal nerve (broken line). Same experiment as in Fig. 10.

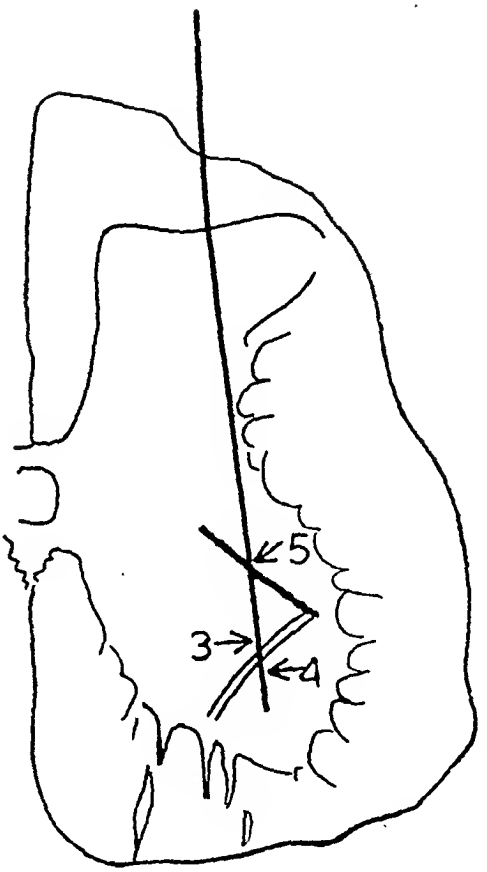

Fig. 35. Schematic picture illustrating the interpretation of the threshold curve of the deep peroneal nerve in Fig. 34.

In their general course the curves of the deep peroneal nerve are similar to those of the gastrocnemius nerve. This shows that the motoneurons of both nerves are similarly distributed in the transversal plane of the spinal cord. There are nevertheless some characteristic differences which will now be described.

A. Cell Bodies and Axons.

Fig. 34 shows an experiment in which the electrode passed just medial to the lateral cell bodies (the same experiment as in Fig. 10). The minimum value for the gastrocnemius nerve (solid line) at arrow 1 corresponds to the activation of a bundle of axons, whose cell bodies are situated just lateral to point 1 in the cross section (see p. 24). The curves of the deep peroneal nerve (broken line) has no minimum at the same place as that of the gastrocnemius nerve, a fact indicating a difference in the position of the cell bodies of the two nerves. Instead the curve of the deep peroneal nerve has two dips (at arrows 5 and 3—4), which might indicate the activation of two separate bundles of axons. These dips are situated on either side of that of the gastrocnemius nerve. Thus one bundle of axons might run (through point 5) dorsal and medial, and the other (through points 3—4) ventral and lateral to the bundle of axons for the gastrocnemius nerve. This interpretation has not been confirmed, however, in experiments, in which the electrode has crossed the axons medially in the anterior column. Fig. 36 illustrates such an experiment (the same as in Fig. 6). The curve of the gastrocnemius nerve (solid line) shows a pronounced dip (arrows 1 and 2), representing the activation of a distinct bundle of axons (see p. 23). The corresponding part of the deep peroneal nerve (broken line) shows a slowly falling course, which in all probability does not represent the activation of axons but instead that of a dense collection of dendrites spread over the mediodorsal part of the anterior column (cf. below). Judging by this experiment the axons of the deep peroneal nerve must be situated lateroventrally of the axons of the gastrocnemius nerve. Hence, the cell bodies of the first mentioned nerve must be situated ventral to the latter. These observations show that only the dip at arrows 3—4 in Fig. 34 corresponds to the activation of the axons of the deep peroneal nerve. These axons emerge from cell bodies situated lateral to points 3—4 in the cross section, *i. e.* ventral to the cell bodies of the gastrocnemius nerve. Other experiments have confirmed this interpretation. The dip at arrow 5 (the deep peroneal nerve) in Fig. 34 may correspond to the activation of a bundle of dendrites running dorsomedially. The schematic picture in Fig. 35 illustrates this interpretation.

Fig. 37 shows an experiment from the same animal as in Fig.

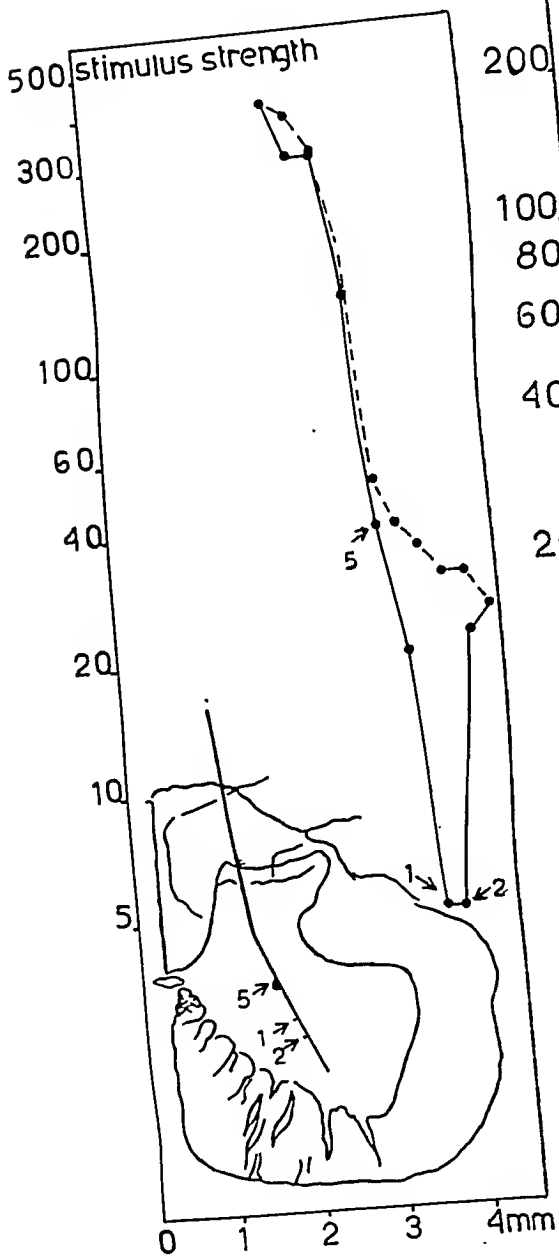

Fig. 36. Threshold curves for the gastrocnemius nerve (solid line) and the deep peroneal nerve (broken line). Same experiment as in Figs. 6 and 26.

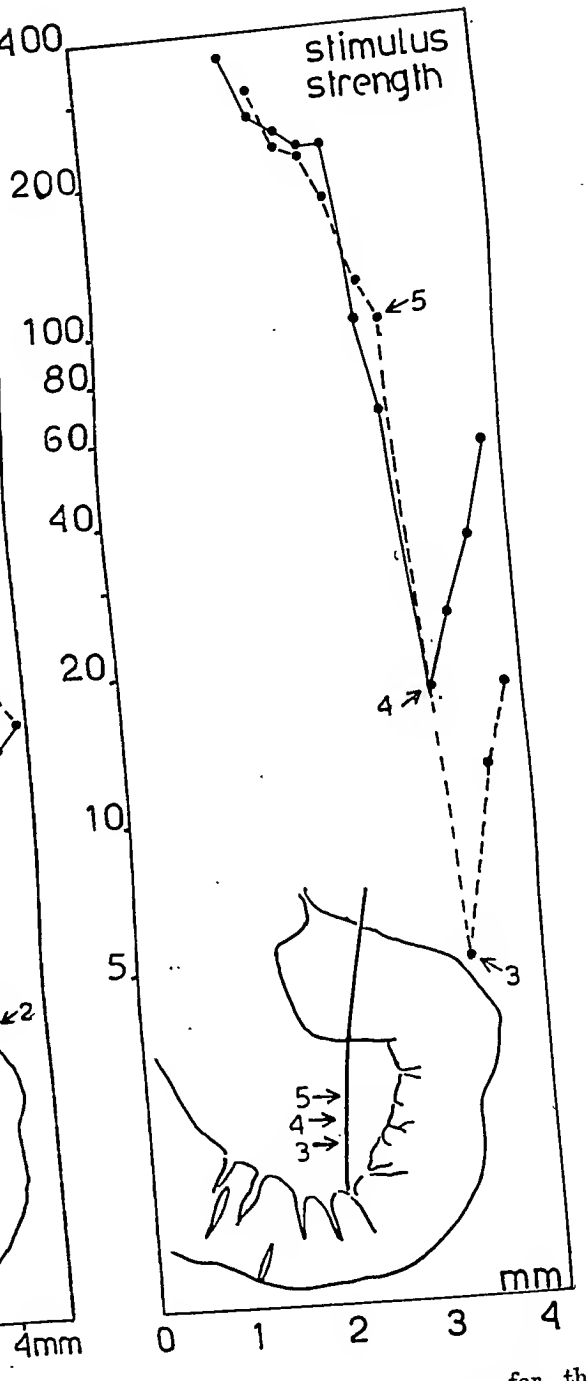

Fig. 37. Threshold curves for the gastrocnemius nerve (solid line) and the deep peroneal nerve (broken line). Same animal, side and segmental level as in Fig. 36.

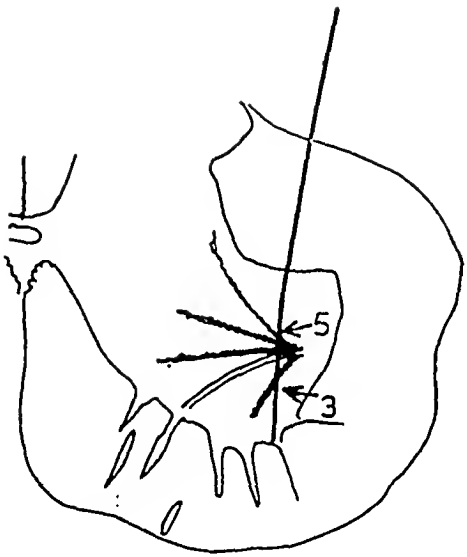

Fig. 38. Schematic picture illustrating the interpretation of the threshold curve of the deep peroneal nerve in Fig. 37. Full description in text.

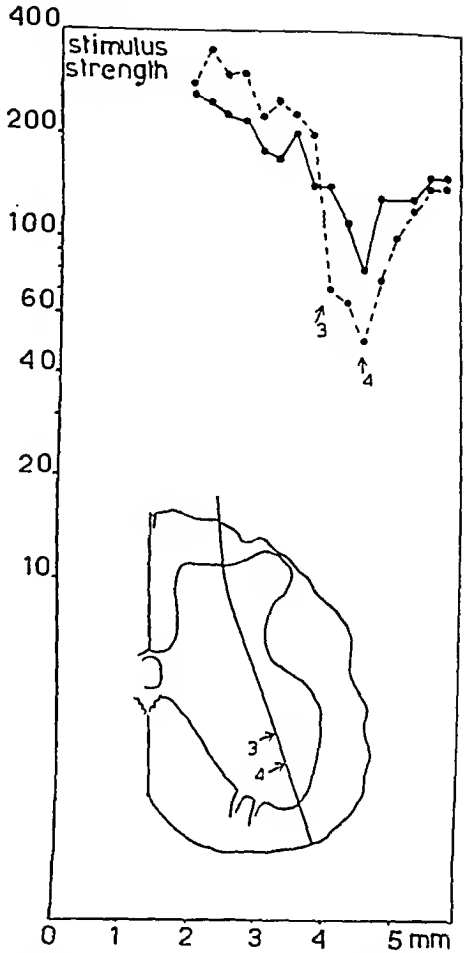

Fig. 39. Threshold curves for the gastrocnemius nerve (solid line) and the deep peroneal nerve (broken line). Same experiment as in Figs. 21 and 29.

36. The needle was inserted on the same side and at the same level, though more lateral. The curve of the deep peroneal nerve (broken line) has a pronounced dip as opposed to the one in Fig. 36. The steep fall between the values at arrows 5 and 3 represents the activation of a bundle of elements collected densely together and situated in the lateral part of the anterior column (between points 5 and 3 in the cross section). It is obvious that some of these elements are made up of axons originating in cells just lateral to the channel. The cross section shows, however, that the needle was in such a position that it must also have intersected the majority of the dendrites of these motoneurons close to their origin in the cell bodies. The diagram in Fig. 38 illustrates this

interpretation. After passing a region with only a few dendrites (dorsal to point 5 in the cross section), the needle struck the processes (dendrites and axons) close to their origin in the cell bodies and before they spread over a considerable surface. The curve in Fig. 34 has another shape and a less steep fall. This is due to the needle running through the lateral part of the posterior column, which contains dendrites dorsally (see p. 42), and also to it being situated more medially of the cell bodies than it is in Fig. 37. In consequence of this their processes have been intersected over a longer stretch in Fig. 34 than in Fig. 37. Further it is interesting to see that in Fig. 37, the position of the needle in relation to the deep peroneal motoneurons resembles that of the electrode in relation to the gastrocnemius motoneurons in Fig. 12. In consequence corresponding parts of the curves have similar shapes.

Fig. 39 shows an experiment (the same as in Fig. 21 and 29) in which the needle, as in Fig. 37 passed through the lateral part of the anterior column but took another direction. The rapid decrease in the curve of the peroneal nerve (broken line) down to the value at arrow 3 indicates the existence of dendrites and axons emanating from cell bodies lateral or dorsolateral to point 3 in the cross section. These cell bodies of the deep peroneal nerve evidently have a position similar to that shown in connection with Fig. 37. Like the curve for the gastrocnemius nerve in the same experiment, the part of the curve to the right of arrow 3 shows the existence of dendrites, which run across the lateral part of the anterior column to the ventral part of the lateral funiculus (see p. 37).

B. Dendrites.

The shape of the curves shows that the dendrites of the deep peroneal motoneurons have the same course as those of the gastrocnemius motoneurons. Only a few details of particular interest will be discussed here.

Fig. 36 illustrates an experiment in which the electrode passed through the anterior column between the cell bodies and the anterior commissure. It has already been pointed out that the curve of the deep peroneal nerve (broken line) to the right of arrow 5 represents the activation of dendrites spread across the mediodorsal part of the anterior column. Arrow 5 marks the

value at which the steep fall of the curve passes into the slightly sloping part. It is clear that this value indicates the dorsal boundary of the commissural dendrite bundle (situated at about point 5 in the cross section). It is interesting to notice that the curve of the gastrocnemius nerve (solid line) also has a slight deviation at arrow 5, which indicates activation of the commissural bundle for this nerve too. The last part of this curve, however, is dominated by the deep dip, representing activation of the motor axons. As other experiments (Fig. 5 and 16) show that this part, *i. e.* the mediodorsal part of the anterior column, contains a dense collection of dendrites belonging to the gastrocnemius motoneurons, it is obvious that the motor axons "conceal" the presence of these dendrites. It is probable that the curve of the gastrocnemius nerve to the right of arrow 5 would have looked the same as that of the deep peroneal nerve, had the corresponding area of the spinal cord only contained dendrites (as in the case of the deep peroneal nerve).

Fig. 23 shows an experiment in which the needle penetrated the lateral funiculus. The curves show that the lateral funiculus contains dendrites belonging to the two nerves. To the left of arrow 2 each curve has two dips, which shows that the dendrites are not evenly spread over the corresponding area dorsal to point 2 in the cross section, but that they are destined to the lateral funiculus in the form of bundles. It is particularly interesting to see that the dips of the curves do not coincide, a fact indicating that the dendrites of the two nerves may be directed towards different parts of the funiculus. To the right of arrow 1 the curves have a common minimum, which shows that the dendrites of both nerves within the ventral part of the lateral funiculus are comparatively plentiful (cf. p. 39).

Different examples of the course of dendrites belonging to the motoneurons of the deep peroneal nerve are to be found in the following: Fig. 15 (commissural dendrites), Fig. 39 (lateroventral), Fig. 34 (posterior), Fig. 25 (dendrites to the lateral funiculus) and Fig. 30 (contralateral dendrites). These examples show that the threshold values corresponding to the activation of dendrites are usually lower in the curves of the deep peroneal nerve than in the gastrocnemius nerve. This observation has been confirmed in other experiments. The question must be left open for the present, as to whether this indicates a difference in the density or in the properties of the two nerves.

When summing up the following may be said concerning the intraspinal course of the deep peroneal motoneurons in the cross section. The cell bodies are situated in the lateral part of the anterior column ventral to those of the gastrocnemius nerve. From these cell bodies there branch off, firstly axons running towards the ventral surface of the spinal cord, and secondly dendrites with a course similar to that of the gastrocnemius nerve. Hence, the dendrites can be divided into *medioventral*, *lateroventral*, posterior and commissural groups. They may be found in greater numbers here than in the gastrocnemius nerve.

Discussion.

The above experiments have shown that direct volleys can be evoked by the local activation of various parts of motoneurons, *i. e.* axons, cell bodies and dendrites. The latencies for the direct volleys evoked from the dorsal part of the cord are longer than the latencies for those from the ventral part (see above p. 14). Since the differences do not exceed 0.3 msec. and since no sudden shortenings of the latencies were observed in the experiments when the needle was pushed ventrally, it is not likely that presynaptic elements were stimulated in the dorsal part (unless there is a synaptic delay less than 0.1 msec.). Therefore it may be suggested that the differences in the latencies concerning the direct volleys are due to the conduction of the impulses in the dendrites of the motoneurons.

In experiments on the oculomotor nucleus LORENTE DE NÓ (1935 f) proved that the soma of the motoneurons has a lower threshold than the axon. On account of this it is worthy of note that direct volleys in the experiments described above can be produced by activation of motor axons with low stimulus strength, while areas containing motor cell bodies require high strength. As a rule, however, the preparations show that the motor cell bodies in the close vicinity of the channel are injured, and it is therefore probable that the high threshold values are attributed to this injury. Thus, an electrode of the size, which often is used in experiments of this kind, easily injures the cell bodies before the needle tip is sufficiently close to be able to activate them. This observation is in agreement with the experience, which shows that it is also difficult to record potentials from the motor cell bodies with a microelectrode (LORENTE DE NÓ, 1939, LLOYD 1942).

When activating areas containing dendrites their density has been evaluated on the basis of the threshold values obtained. To a certain extent this assumption implies a simplification of

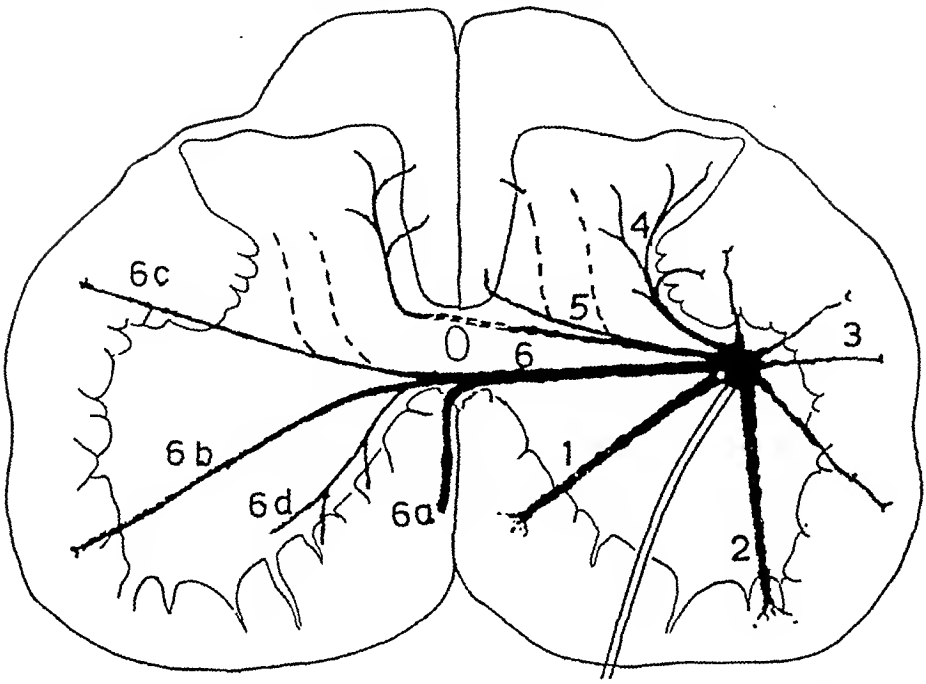

Fig. 40. Schematic diagram showing the position of cell bodies, axons and dendrites belonging to the gastrocnemius motoneurons in a cross section of the seventh lumbar segment of the spinal cord. The dendrites are divided into the following groups: 1) medioventral, 2) lateroventral, 3) lateral, 4) laterodorsal, 5) mediodorsal, 6) anterior commissural, subdivided into four groups (a—d). Broken line represents a hypothetical course.

the analysis. Since the dendrites are thick near the cell body and then taper towards the periphery, the different parts of these elements probably possess varying excitability properties. In the experiments described, however, the electrode has usually intersected the dendrites more or less perpendicularly, *i. e.* at about the same distance from the cell bodies in each case. Further the curves have usually been interpreted separately. Consequently it may be assumed that even taking into consideration the possibility of differences in the excitability of the dendrites within different regions, it will not alter the conclusions already drawn with regard to the distribution of the dendrites.

Fig. 40 shows a schematic diagram of the analysis of the positions of the cell bodies, the axons and the dendrites belonging to the gastrocnemius motoneurons in a cross section of the middle part of the seventh lumbar segment of the spinal cord. The cell bodies of the gastrocnemius nerve are to be found in the dorsolateral part of the anterior column. As opinions differ somewhat as regards the configuration of the cell columns in cat, no attempts

have been made in this work to correlate the findings to the cell columns. The cell bodies of the deep peroneal nerve also form one single group, which is situated ventral to the cell bodies of the gastrocnemius nerve. These two groups are to be found dorsally among the lateral groups of cell bodies. This fact confirms the generally accepted view, *i. e.* that motor cell bodies for the limb musculature are situated among the lateral cell groups, and also that the cell bodies for the distally situated muscles of the extremities are to be found dorsally among the lateral groups (see *e. g.* Bok 1928).

Moreover, the investigations have shown that the axons of the gastrocnemius nerve from the cell bodies run in a well collected bundle towards the ventral surface, and that this bundle leaves the spinal cord on the side in which their cell bodies are situated. It cannot be excluded that some few axons take another course, say, through the anterior commissure or the posterior roots. The experiments show, however, that a well collected bundle does not exist in any other area but what is described in connection with Fig. 13 and 14.

The dendrites of the motoneurons described spread over extensive parts of the spinal cord. They are most dense within the ventral part of the side of the spinal cord containing their cell bodies. The distribution of the dendrites according to Fig. 40 calls to mind that, indicated by Cajal (1909). In this work, however, it has been possible to investigate the dendrites of motoneurons with different functions and follow them over greater stretches to definite regions. Moreover, the investigations have been carried out in experiments on living and adult animals as opposed to the histological, which are usually based on embryos or newborn animals.

Consideration has only been taken to the distribution of the dendrites in the cross section. It is not likely that the presence of the axial dendrites, mentioned by Cajal (1909), can alter the general picture represented in Fig. 40.

Fig. 40 shows that the dendrites are directed towards different areas of the white matter. It is obvious that this regular distribution indicates the position of afferent collaterals from different spinal tracts, which either direct or via internuncial systems transfer the activity to the motoneurons. The main part of the dendrites runs towards the anterior and lateral funiculi. It is probable that these systems unite either directly or indirectly

with the propriospinal and bulbospinal tracts, which are situated within these regions (SHERRINGTON and LASLETT 1902, 1903, LLOYD 1941 a, 1942, 1944). It is also probable that a smaller collection is directed towards the collaterals of the propriospinal fibres into the deepest part of the posterior funiculus. It must be noted that it has not been possible in these experiments to show any bundle of dendrites running towards the dorsomedial part of the posterior column, *i. e.* the spot where the intrasegmental afferent collaterals from the proprioceptive nerves enter the grey matter of the spinal cord. This confirms the assumption, made by CAJAL (1909), that "les collatérales réflexo-motrices" land mainly on the surface of the motor cell body and not on the dendrites (cf. KAPPERS 1920). As regards the contralateral dendrites it may be suggested that the bundles 6 a and c in Fig. 40 are united either directly or indirectly with the collaterals from the lateral and ventral corticospinal tracts.

It is obvious that the dendrites not only increase the synaptic surface of the motor cells but also contribute to a segregation of the different functional groups of axon endings, which land on the surface of the motoneurons (cf. BODIAN 1942).

Since it is known that not all the motoneurons in the anterior column have dendrites with extensive expansion (CAJAL 1909), a differentiation may also be possible in the case of motor cells belonging to the same nerve (*e. g.* the gastrocnemius nerve) by means of which a functional differentiation of a group of motoneurons would be possible.

In some cases the experiments show clearly that indivudual variations may occur concerning the distribution of the dendrites to different areas. Since it is known that the spinal tracts may vary in size (SHERRINGTON and LASLETT 1902 as regards the dorsal funiculus) and distribution (BOK 1928 as regards the pyramidal tracts), it is possible that these variations in dendrites and spinal tracts may be correlated.

In the experiments described in this work volleys due to transynaptic stimulation have also been studied systematically. The results from these observations will, however, be published later on. Here it will only be mentioned that the position shown by the premotor elements confirms the conclusions drawn concerning the distribution of the dendrites.

Summary.

1) Experiments have been performed on cats, in which electrical microstimulation in the middle of the seventh lumbar segment of the spinal cord was used. A needle electrode isolated down to the tip (diameter not exceeding 0.03 mm) was pushed through the spinal cord with the help of a micrometer gauge, and the relative threshold values for the direct potential waves, recorded from the gastrocnemius and deep peroneal nerves of both sides, were measured at different depths of the stimulating needle point. The position of the channel left behind by the needle electrode was reconstructed with the help of histological sections.

2) The experiments show that local stimulation of different intraspinal parts of the motoneurons is possible.

3) The position of the motoneurons belonging to the two nerves has been established by making a comparison between, firstly the threshold values obtained when activating different regions in the same experiment, and secondly between the distribution of the threshold values in different experiments. The result of this analysis is to be seen in the schematic picture in Fig. 40 (p. 61), which shows the position of the axons, the cell bodies and the dendrites of the gastrocnemius motoneurons. The cell groups of the gastrocnemius nerve is situated in the dorsolateral part of the anterior column. Motor axons branch off from here, running in a dense bundle towards the ventral surface of the spinal cord on the same side. From the cell group for the deep peroneal nerve, situated ventral to the gastrocnemius cell bodies, there branches off a bundle of axons, which runs through the anterior column ventral and lateral to the gastrocnemius axons. The dendrites of these two nerves are most dense in the ventral part of that side of the spinal cord, which contains their cell bodies. The dendrites of the deep peroneal motoneurons may possibly be greater in number than those of the gastrocnemius motoneurons. Their general distribution, on the other hand, is the same.

According to Fig. 40 the dendrites of the motoneurons studied are divided into the following groups: 1) *medioventral* running to the anterior funiculus, 2) *lateroventral* to the ventral part of the lateral funiculus, 3) *lateral* to the middle and the dorsal part of the lateral funiculus, 4) *laterodorsal* to the lateral part of the posterior column and to the adjacent part of the lateral funiculus, 5) *mediodorsal* running to the deepest part of the posterior funiculus and possibly even through the posterior commissure to the medial part of the posterior column on the other side, 6) *anterior commissural dendrites* running through the anterior commissure to the other side of the cord, there dividing into several collections: a) a strongly developed one to the medial part of the anterior funiculus, b) a less developed one (lateroventral) running to the ventral part of the lateral funiculus, c) a still less developed one (laterodorsal) running to the dorsal part of the lateral funiculus and d) a faint one running inside the boundary between the anterior column and the anterior funiculus.

4) The lowest threshold values are obtained when activating the area containing the motor axons. Stronger activation, however, is required to produce direct volleys from the area containing motor cell bodies. It is evident that the cell bodies are usually destroyed before the needle is sufficiently near to be able to activate them.

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THE NATURE AND OCCURRENCE OF PRESSOR AND DEPRESSOR SUBSTANCES IN EXTRACTS FROM BLOOD VESSELS

By

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Stockholm 1948

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PREFACE

The main part of this investigation has been carried out in the Department of Physiology and Pharmacology, Kungl. Veterinärhögskolan, Stockholm.

It is my pleasure to have this opportunity for expressing my sincere gratitude to Professor YNGVE ZOTTERMAN, Head of this Department, for his continual interest and neverfailing support in bringing this work to completion and for his encouragement and advice during all phases of this investigation. I also wish to express my thanks to him as my teacher and personal friend.

When serving as an assistant in the Department of Physiology, Karolinska Institutet, Stockholm, I did some preliminary work on this subject and also had the great favour of working with my former teacher and Head of that Department, Professor U. S. VON EULER. During the course of this investigation I have had the invaluable opportunity of obtaining his advice concerning many questions dealing with this problem. For this I desire to express my deepest gratitude.

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placing his time at my disposal in preparing some of the substances used in this investigation and for his help with some of the chemical problems.

To my assistant, Miss B. GREDBORN, I wish to take this opportunity to express my deep gratitude. With great care, interest and discernment she has helped me with this work and offered much of her private time in finishing extraction procedures and experiments of long duration. I am also indebted to her for her assistance in preparing the manuscript.

The English text has been revised by Mr. W. S. MONLUX and for his conscientious work I wish to express my sincere thanks.

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And, finally, I desire to thank my wife for the encouragement and support she has always given me in my work.

Stockholm, October 1948.

CARL G. SCHMITERLÖW

GENERAL INTRODUCTION

»It is well to recognize at the outset that when any particular organ is stimulated by way of its sympathetic nerves not only are the proper cells of that organ affected but also the smooth-muscle cells of the blood vessels distributed to the organ».

(CANNON and ROSENBLUETH: »Autonomic Neuro-Effector Systems», 1937, p. 62.)

Ever since the discovery that adrenaline mimics the effects of sympathetic nerve action many investigations have been carried out concerning the physiological rôle of adrenaline in relation to the transmission of sympathetic nerve impulses. ELLIOTT (1904) was the first to suggest that adrenaline may be released at the sympathetic nerve endings. The classical work of OTTO LOEWI in 1921, concerning the existence of a "Vagusstoff" and a "Sympaticusstoff", founded the modern conception of autonomic transmittor substances. The extensive works of BACQ, CANNON and ROSENBLUETH and their co-workers gave new evidences about the existence of a sympathetic "mediator" which they called "sympathin" in order to separate it from the adrenaline produced in the adrenal glands. These investigations led CANNON and ROSENBLUETH to state: "Since the chief manifestations of a nerve impulse is electrical, it was natural to infer that the terminal organs are acted upon physically. We now have evidence, however, that a chemical step intervenes between the phenomena of the nerve and those of the effector" ("Autonomic Neuro-Effector Systems", 1937).

By stimulating different sympathetic nerves a sympathomimetic substance was released into the blood stream which, however, in certain respects differed from adrenaline. The chemical nature of this substance has been widely discussed.

Through the work of U. S. VON EULER, during recent years, evidence has been brought forth concerning the nature of the sympathomimetic substance present in extracts from most organs, including adrenergic nerves. These investigations have founded a wider and more exacting knowledge about the occurrence and physiological rôle of this substance. The methods employed by him opened new fields of research concerning the sympathetic mediator.

It was pointed out previously by CANNON and ROSENBLUETH that at least some part of the "sympathin" released by electrical stimulation of the sympathetic nerves to any particular organ may originate from the smooth muscles of the blood vessels. The same argument also holds true for the sympathomimetic substance present in extracts from different organs.

The present investigation deals with the nature and occurrence of a sympathomimetic substance in extracts from blood vessel walls and also with the nature and occurrence of a blood pressure reducing and smooth muscle stimulating agent in these extracts. The investigation was undertaken in the hope that the results might add to our knowledge concerning the physiology and pharmacology of the sympathetic nervous system.

PART I

HISTORICAL SECTION

Chapter I

The chemical mediation of sympathetic nerve impulses

In 1895, OLIVER and SCHÄFER published their thorough investigations of the physiological action of suprarenal extracts. These extracts contained an active principle causing, when injected intravenously, an acceleration of the heart, a rise in blood pressure, a constriction of the intestinal blood vessels, a temporary shallowing or cessation of the respiration, a dilatation of the iris and an inhibition of the intestine and the bladder. These findings were confirmed by SZYMONOWICZ and CYBULSKI (1896), LEWANDOWSKY (1899, 1900) and BORUTTAU (1899). LEWANDOWSKY called attention to the possible significance of the observation that the action of the suprarenal extracts upon plain muscle simulates that of electrical excitation of the sympathetic nerves supplying each particular muscle. This intimation seems to be the first attempt to approach the more general rule concerning a possible relationship between sympathetic nerve impulses and some humoral factor. LEWANDOWSKY's original suggestion was further strengthened by the observations made by LANGLEY (1901). He studied the effects of the active principle of the suprarenals on different glands, bile secretion, gall bladder, pancreatic secretion, the eye, heart and blood vessels, intestine, oesophagus and stomach, rectum and anus, urinary bladder, internal and external generative organs and respiration. "It is", LANGLEY says, "note-worthy that the effects produced by suprarenal extract are almost all such as are produced by stimulation of some one or other sympathetic nerve. In many cases the effects produced by the extract and by electrical stimulation of the sympathetic nerve correspond exactly. The view which

obviously presents itself to account for these facts is that the suprarenal extract has a specific stimulating action on sympathetic nerve endings . . ."

After LANGLEY's work, in 1901, the definite isolation and structural elucidation of the active principle of the suprarenal glands was made by TAKAMINE (1901). This discovery made it easier to perform quantitative determinations of the effects of the active principle of the suprarenal glands. TAKAMINE named this active principle "adrenalin".

Using adrenaline, BRODIE and DIXON (1904) showed that this substance did not constrict the blood vessels of the lungs, a fact which confirmed the earlier findings that "suprarenal extract varies in its action on unstriated muscle, some it causes to contract, some it inhibits, and on some it has little or no effect" (LANGLEY, 1901).

In 1904, ELLIOTT published a short communication in which he states that an adrenalectomized animal when moribund, "exhibits symptoms that are referable to a hindrance of the activities of these tissues especially that are innervated by the sympathetic". From this observation he drew the conclusion that the maintenance of sympathetic nerve activity is due to the presence of the adrenaline (or its immediate precursor) secreted by the suprarenal glands. ELLIOTT also made another observation of greater interest and significance. After complete denervation the plain muscle of the dilatator pupillæ still responded to adrenaline, even better than the iris whose nervous relations were intact. This led him to suggest that the site of action of adrenaline might be a sort of mechanism "developed out of the muscle cell in response to its union with the synapsing sympathetic fibre, the function of which is to receive and transform the nervous impulse". ELLIOTT concludes his short communication with the words: "Adrenaline might then be the chemical stimulant liberated on each occasion when the impulse arrives at the periphery". This statement is the first direct suggestion that sympathetic nerve activity might include the release of a highly active principle at the nerve endings — a "humoral transmission".

In 1905, ELLIOTT extended his experimental research concerning the action of adrenaline, and was able to show that in all vertebrates the action of adrenaline on any plain muscle mimics closely the effect following excitation of the sympathetic nerves supplying that muscle. As he found that neither the sympathetic nerve cells with their fibres nor the contractile muscle fibres were irritated by adrenaline, he suggested that the stimulation takes place at the junction of muscle and nerve, where a special substance is stimulated by adrenaline. Furthermore he suggested that the motor or inhibitory effect upon a cell depends upon the nature of this substance.

This latter hypothesis was critically reviewed by LANGLEY (1905) who found it difficult to believe that this hypothetical substance, which is acted upon by adrenaline, is developed from the muscle in consequence of its union with a sympathetic fibre (the myo-neural junction). His chief argument was that the section of the sympathetic nerves did not cause any atrophy of this substance. He agreed with ELLIOTT in regard to the presence of receptive substances but thought it to be a constituent of the cell itself. He also considered "that a cell may make motor or inhibitory receptive substances or both, and that the effect of a nervous impulse depends upon the proportion of the two kinds of receptive substance which is affected by the impulse".

As will be seen later this hypothesis has much in common with the view advanced by CANNON and ROSENBLUETH about excitatory and inhibitory sympathin.

The same idea concerning the liberation of specific hormones from excited nerves was presented by DIXON and HAMILL (1909). Proceeding from the view adopted by, amongst others, LANGLEY that drugs induce specific effects by combining with receptive substances in living protoplasm, they studied the mode of action of secretin and made some general conclusions as to the action of drugs. Judging from analogy they thought it reasonable to believe that "excitation of a nerve induces the local liberation of a hormone which causes specific activity by combination with some constituent of the end organ, muscle or gland".

Although many authors had thus theorized about the rôle of humoral factors in the transmission of nervous impulses to effector organs it was not until 1921 that LOEWI gave the experimental evidence for the correctness of these theories. His experiments, now regarded as classical, proved the existence of specific substances liberated from cardio-inhibitor as well as cardio-accelerator nerve fibres on stimulation. That the occurrence of such specific ergones was not only limited to the heart was shown the same year by CANNON and URIDIL (1921). They found that stimulation of the hepatic nerves caused a discharge of a substance from the liver, which caused an elevation of the blood pressure and an increased heart rate and in 1922, CANNON and GRIFFITH proved that this released substance was actually carried in the blood stream.

After these decisive findings a research was commenced which aimed at the identification of and a more intimate knowledge of the nature of these neuro-hormones. LOEWI's "Vagusstoff" was soon identified as acetylcholine. The chemical and physiological nature of the sympathetic transmittor substance was, however, not so easily determined.

Soon after the work of LOEWI several authors pointed out the close resemblance between the sympathetic mediator and adrenaline. Stimulation of the sympathetic nerves to different organs caused a release of a sympathomimetic substance, the effect of which could be studied on test organs which had previously been disconnected from the central nervous system. The released sympathetic substance was carried to the indicator by the blood stream and if the stimulated organ was also disconnected from the central nervous system and both adrenals rendered inactive it could be anticipated that the effect on the test organ could only be due to the presence of a humoral sympathomimetic factor originating from the stimulated organ. Many such experiments were performed (CANNON and URIDIL, 1921, CANNON and GRIFFITH, 1922, BRINKMAN and VAN DAM, 1922, KAHN, 1926, RYLANT, 1927, RYLANT and DEMOOR, 1927, LANZ, 1928, FINKLEMAN, 1930, CANNON and BACQ, 1931, CANNON and ROSENBLUETH, 1933,

CATTELL, WOLFF and CLARK, 1934, ROSENBLUETH and MORISON, 1934 etc.). It became evident that the liberated substances — from liver, heart, intestine, uterus, bladder, vascular and pilomotor muscles, aqueous humor, salivary glands — exerted on the test organs — denervated heart, nictitating membrane, intestine, retractor penis, uterus, iris etc. — effects very similar to those of injected adrenaline. The view that the sympathetic substance was identical with adrenaline was further strengthened by the following facts:

The test organs were rendered more sensitive to the liberated substance through sympathetic denervation or through a previous injection of cocaine (ROSENBLUETH and SCHLOSSBERG, 1931). The effective agent was destroyed when mixed with eosine and exposed to ultraviolet light (LOEWI and NAVRATIL, 1926) and it was also rendered inactive when exposed to air or heat (LANZ, 1928). The effective agent gave the Viale colour reaction (BACQ, 1933) and the green fluorescence reaction of GADDUM and SCHILD (1934) with strong alkali (LOEWI, 1936). Its ultraviolet absorption spectrum was very much the same as that of adrenaline (BACQ and HENRI, 1933, BACQ, HENRI and SCHEPERS, 1933). In this connection it must, however, be emphasized that the specific fluorescence reaction was carried out on perfusates from the frog heart.

Although the general opinion favoured the view that adrenaline and the substance set free by stimulation of some part of the sympathetic system were identical it was suggested by CANNON and BACQ (1931) that the sympathetic mediator be called "sympathin" because it has a different source than adrenaline.

The above-mentioned facts pointed to a chemical identity of the sympathetic substance (sympathin) and adrenaline. There were, however, certain facts which did not coincide. In their early work CANNON and URIDIL (1921) noted that the iris was not dilated by the substance discharged from the liver on sympathetic stimulation, whereas adrenaline gave a strong dilatation. Through the works of CANNON and ROSENBLUETH and their

co-workers during the thirthies evidence was brought forth that there existed distinct differences between adrenaline and sympathin. They observed that the effect of the released sympathin on the test organs — the remote effect — was not invariably the same independant of which region of the sympathetic was stimulated. Stimulation of a sympathetic region where the impulses only cause excitatory answers released a sympathin which, carricd by the blood stream, caused only excitatory effects on the test organs. Stimulation of other regions, where the answer is both excitatory and inhibitory, such as the splanchnic area, causes a release of a sympathin which has both excitatory and inhibitory effects on the test organs. In addition to this fact there were other differences: after ergotoxine, adrenaline produces a fall in blood pressure, whereas sympathin (from regions where sympathetic impulses excite the effectors) still causes a rise. Furthermore sympathin simultaneously released from two different sources gives a combined effect which is much greater than the effect of combined doses of adrenaline which match the separate sympathin action.

These facts led CANNON and ROSENBLUETH not only to state that sympathin was not identical with adrenaline but also to postulate that there were two kinds of sympathin — excitatory and inhibitory.

It is well known that adrenaline exerts both excitatory and inhibitory actions. Some smooth muscles are stimulated, some inhibited. There is, CANNON and ROSENBLUETH argued, no reason to believe that there are two different types of these cells. They elaborated a hypothesis which involved the presence of a substance within the reacting cells which combines with the sympathetic mediator (M). This hypothetical substance within the reacting cells possesses two different actions, viz. excitatory and inhibitory (E and I). When sympathetic nerves are stimulated the result in the effector cells will be ME in a contracting and MI in a relaxing muscle. When these two substances (sympathin E and sympathin I, respectively) escape from the affected cells into the

blood stream they will induce excitatory, inhibitory or, when present together, both effects on remote organs; sympathin E and sympathin I thus have one component in common, viz. the transmitter released at sympathetic nerve endings. This transmitter is then given its characteristic type of activity by combining with an excitatory or inhibitory "receptive substance" inside the cell.

Chapter II

The chemical nature of »sympathin«

A. Different theories

As has been pointed out, the close resemblance between the effect of sympathetic stimulation and the action of intravenously injected adrenaline led many authors to believe that the sympathetic mediator was adrenaline. Furthermore, the "Sympaticusstoff" of LOEWI was found to behave, biologically as well as physically and chemically, like adrenaline; but from the experimental results with released "sympathin" it became evident that this was at least not the whole truth.

ROSENBLUETH (1932) advanced the theory that the intermediary sympathetic substance (the "M" of CANNON and ROSENBLUETH) was the equivalent of adrenaline. The receptor substances of the effector cells, "E" and "I", then determine the action of this liberated adrenaline.

BACQ (1933) pointed out the possibility that several other catechol amines, such as nor-adrenaline (demethylated adrenaline) and β-(3, 4-dihydroxyphenyl)-ethylamine, could serve as mediators in the sympathetic system, and later (1934) he advanced the hypothesis that sympathin I corresponded to adrenaline whereas sympathin E was nor-adrenaline. In this connection some earlier investigations concerning adrenaline-related amines may be briefly quoted.

LOEWI and MEYER (1905) investigated some ketones of the general formula $(OH)_2C_6H_3 \cdot CO \cdot CH_2N\,R_1R_2$ and the corresponding secondary alcohols $(OH)_2C_6H_3 \cdot CHOH \cdot CH_2N\,R_1R_2$ and found that in most cases the reduction of the ketones to secondary alcohols greatly intensified the adrenaline-like action. This increase in activity following the reduction was, however,

according to DAKIN (1905), not a generally obtained phenomenon. Substituting the aminogroup with more complicated radicles, no such increase of activity on reduction occurred. All of the active compounds examined by these and other authors were catechol derivatives, and, as a matter of fact, DAKIN concluded that the catechol nucleus was necessary to produce substances with an adrenaline-like action, since catechol itself was found to cause a rise in blood pressure whereas the base methylamino-ethanol $CH_2(OH)\ CH_2\ NHCH_3$ was without action.

This conception was revised by BARGER and DALE (1910) who in their classical study thoroughly investigated the relationship between the chemical structure and "sympathomimetic" action of a great number of amines. As a general quantitative index of the activity of these substances they used the effect on arterial blood pressure, but, in addition, they also studied the effects on the activity of other organs, such as the isolated non-pregnant cat's uterus. They reached the conclusion that approximation to adrenaline in structure is, on the whole, attended with an increasing intensity of sympathomimetic activity. They also found that of the catechol bases (*i. e.* the closest adrenaline-related amines) those with a methylaminogroup, including adrenaline, reproduce stronger inhibitor sympathetic effects than motor effects and that the opposite was true with the primary amines of the same series. Among the 12 catechol bases which they tested the amino-ethanol-catechol exerted the strongest action on blood pressure, followed by methylamino-ethanol-catechol (adrenaline), the ratio between these two being about 1.5: 1. On testing the action of the various catechol bases on non-pregnant cat's uterus it became evident that the methylamino-bases had a far more pronounced inhibitory effect than any of the others. Thus the amino-ethanol-catechol did not exert any visible action on the tone and rhythm of the uterus whereas its corresponding methylamino-base, adrenaline, caused a marked relaxation. Furthermore, it was observed that after administration of ergotoxine the blood pressure response to adrenaline was reversed, the amino-base still showing a remnant of pressor action.

BARGER and DALE found ELLIOTT's suggestion in regard to the liberation of adrenaline at the sympathetic nerve endings questionable because the action of some of the other bases, particularly of the amino- and ethylamino-bases of the catechol group, corresponded more closely to that of sympathetic stimulation than does that of adrenaline. They also doubted the suggestion made by DIXON and HAMILL that the action, not only of sympathetic nerve impulses, but also of drugs reproducing these effects, is due to the liberation of adrenaline, since they found it unreasonable to believe that *e. g.* the action of amino-ethanol-catechol should involve the liberation of its homologous, methylated and not more active neighbour adrenaline.

The theory advanced by BACQ that nor-adrenaline (amino-ethanol-catechol) was identical with sympathin E received further support through the investigations of STEHLE and ELLSWORTH (1937) who found that the effect of nor-adrenaline upon the blood pressure of ergotaminized decapitate cats mimics closely the effect observed by CANNON and ROSENBLUETH to result from the stimulation of the hepatic nerves. From this fact STEHLE and ELLSWORTH found it likely that nor-adrenaline may be the substance which is liberated when the hepatic sympathetic nerves are stimulated. They admit that their experiments do not throw any light on the question whether nor-adrenaline is sympathin E or if it is only the mediator which has to combine with a cellular substance to form the final sympathin E.

PINKSTON, GREER, BRANNON and BAXTER (1937) made a comparison of the responses of smooth muscles to d, l-nor-adrenaline, l-adrenaline, and liver sympathin and found a better agreement between the action of liver sympathin and nor-adrenaline than between liver sympathin and adrenaline.

Studying the effects of two sympatholytic dioxane compounds, F 883 and F 933, MELVILLE (1937) found that unlike the blood pressure effect of adrenaline, that of nor-adrenaline and splanchnic nerve stimulation (adrenals tied off) are not reversed after injection of these two substances and he thought that this fact

strengthened the possibility that nor-adrenaline may be the physiological chemical mediator of sympathetic nerve endings.

In a continuation of their earlier investigations PINKSTON, GREER, BAXTER and BRANNON (1938) again pointed out the close resemblance between nor-adrenaline and "liver sympathin". They suggested that there were at least two "adrenergic" mediators involved in the transmission of impulses from sympathetic nerves to effector cells. One of these mediators, S_c (= contracting substance), has a greater intrinsic power of inducing contraction but a lesser intrinsic power of inducing relaxation than the other mediator, S_r (= relaxing substance). Their hypothesis differs from the sympathin E and sympathin I theory of CANNON and ROSENBLUETH in certain respects:

1. C. and R. propose a single, undifferentiated mediator, M, presumably adrenaline, whereas P., G., B., and B. suggest two already differentiated mediators, S_c inducing responses similar to those induced by nor-adrenaline and S_r inducing responses similar to those evoked by adrenaline.

2. C. and R. assume that sympathin E is *purely* excitatory and sympathin I *purely* inhibitory whereas P., G., B., and B. suggest that the effects of S_c and S_r differ only in degree but not in kind.

3. The theory of P., G., B., and B. renders the postulation of the intervention of cellular constituents (the "E" and "I" of C. and R.) unnecessary in order to account for two "sympathins", and they emphasize "that if the *tentative* assumption is made that l-nor-epinephrine is S_c and l-epinephrine is S_r, a simple working hypothesis is afforded for explaining both local and remote "adrenergic" effects induced by stimulation of postganglionic sympathetic fibres . . ."

From a purely theoretical standpoint they also discussed the *possible* origin of nor-adrenaline. If it is tentatively accepted that adrenaline is formed from tyrosine or phenylalanine through oxidation and subsequent monomethylation and decarboxylation, it could be equally well accepted that nor-adrenaline is formed in the same way but without the methylation of the amino group.

Other theories regarding the formation of adrenaline have been suggested and they can equally well explain the formation of nor-adrenaline.

Another theory advanced by BACQ (1934, 1935) that sympathin I is adrenaline and sympathin E corresponds to a partially oxidized adrenaline with only excitatory properties has been critically reviewed by BLASCHKO and SCHLOSSMANN (1936) and CANNON and ROSENBLUETH (1937). The former authors found that at no stage in oxidation of adrenaline is there any difference in inhibitory and excitatory activity.

In 1938, SHAW described a colour reaction which could be used not only for the determination of adrenaline but also for the differentiation between adrenaline and *e. g.* nor-adrenaline. He found that frog's heart contained adrenaline whereas a variety of organs from the rabbit (including intestine, heart, liver, kidney and stomach) gave a colour reaction which was obviously not due to adrenaline but to some other colour-producing substance. Only in the case of rabbit's prostate was there clear evidence that the colour reaction was due to adrenaline (which affords new evidence for the findings of EULER, 1934).

In a study of the activity of dopa decarboxylase, BLASCHKO (1942) was able to show that the primary amine nor-adrenaline could be formed in the body. CANNON and ROSENBLUETH (1935) had raised some objections against the nor-adrenaline-theory since they found it difficult to believe that adrenaline could be demethylated in the body. According to BLASCHKO this demethylation need not be assumed, the primary amine being formed first, the secondary amine arising from it by N-methylation.

B. The investigations of Euler

During a study of the piperidine content of various animal organs, EULER (1945) observed that most organs contained considerable amounts of a pressor substance different from piperidine which had been found as a normal constituent of urine. Purified

extracts gave a rise in the blood pressure of the cat under chloralose anaesthesia, enhanced by cocaine and apparently reversed by ergotamine. They gave an inhibition of the isolated rabbit's intestine and of the non-pregnant uterus of the cat after atropine administration and, furthermore, stimulated the isolated rabbit's uterus and the frog's heart. These extracts also gave colour reactions with ferric chloride just as does adrenaline and a strong Folin-Cannon-Denis' reaction with phosphotungstic acid. These findings indicated the presence of a sympathomimetic substance which in all probability was in some way related to the "Sympaticusstoff" of LOEWI or the sympathin of CANNON and his co-workers.

This preliminary work of EULER was then widely extended. In 1946 (a) he published his observations concerning the presence of a substance having sympathin E properties in extracts from spleen which was specially rich in pressor substance. Using special extraction and purification methods, which will be referred to later on (see Methods), he showed that these purified splenic extracts possessed a pressor activity which was equivalent to about 10 μg adrenaline per g of tissue. This pressor substance increased the heart rate and raised the blood pressure of the chloralosed cat and this pressor action was enhanced by cocaine. The pressor effect was not reversed by ergotamine (contrary to the earlier findings, where the reversal was obviously due to contaminating, blood pressure lowering, substances) and when the extracts were compared with equi-pressor amounts of adrenaline it was found that they also differed in other respects. The extracts did not stimulate the isolated rabbit's uterus to the same extent as adrenaline and the inhibiting effect of adrenaline on the isolated rabbit's intestine and the non-pregnant cat's uterus was more pronounced than that of the purified extracts. Furthermore the extracts had less pupil dilating action than equi-pressor amounts of adrenaline. Not only were there biological differences but the extracts also differed from adrenaline in that they did not give the fluorescence reaction characteristic of adrenaline (GADDUM and SCHILD, 1934). From the results of colour and fluorescence reactions and

biological tests made on purified extracts, EULER drew the conclusion that the pressor substance in these extracts was not identical with adrenaline. From the colour reactions it could be concluded, however, that the active agent was most probably a catechol compound of the same order of activity as adrenaline. EULER also called attention to the similarity between the action of the purified spleen extracts and the postulated sympathin E on the one hand and such amino-bases of the catechol group as noradrenaline and d, l—3: 4-dihydroxy-nor-ephedrine on the other.

In a later work (1946 b) EULER showed that the mammalian heart (cattle, horse and cat) also contained a sympathomimetic substance which in some respects resembled adrenaline but in others differed from adrenaline. The amount of this active substance corresponded to about 5 μg adrenaline per g of fresh tissue in its pressor action. Here there was also a striking parallelism between the active substance and d, l—3: 4-dihydroxy-norephedrine, and the biological effects of the substance agreed well with that of the postulated sympathin E. These facts did not fully coincide with the findings of LOEWI (1936) and of CANNON and LISSÁK (1939). LOEWI investigated the effect of dialyzed extracts from frog hearts on the isolated frog heart and found it to be identical with adrenaline not only in its biological action but also in regard to its fluorescence reaction. This statement, however, refers to frog heart, and it was later shown by EULER (1946, d) that the sympathomimetic substance in extracts from frog heart really seems to be identical with adrenaline. In a later work LOEWI (1937) investigated the "adrenaline-content" of guinea-pig's heart, using his dialyzing extraction procedure and the frog's heart as test object. He found a rather constant content of "adrenaline" in all parts of the heart, amounting to some 0.15 μg per g of tissue, and he also found that "nach Behandlung mit Fluoreszenslicht die Wirkung der Dialysate ausblieb". That CANNON and LISSÁK found the active substance in extracts from cat's heart to be identical with adrenaline is somewhat confusing but some criticism may be advanced in regard to their interpretation of the results (see Discussion). The view of EULER

that the sympathomimetic action of extracts from mammalian hearts was mainly due to the presence of a substance other than adrenaline (small amounts of adrenaline, EULER admits, might be present since there are chromophile cells present around the heart) was supported by the earlier observations of SHAW (1938) and RAAB (1943). Using the arseno-molybdate colour reaction (SHAW) they showed that this colour reaction in heart extracts could certainly only partly be due to the presence of adrenaline.

The early observation of EULER (1945) that extracts from the nerve-free placenta did not exert any sympathomimetic activity suggested that the active substance was in some way connected with nervous structures.

In this connection it is interesting to note that as early as 1907 DIXON and TAYLOR found that alcoholic extracts of human placenta contained substances which on intravenous injection produced effects somewhat similar to that of adrenaline. Only two years later, however, ROSENHEIM (1909) was able to show that the fresh human placenta did not contain any pressor principle and that the activity of the extracts, prepared according to DIXON and TAYLOR, was due to products of initial putrefaction. ROSENHEIM was able to identify several pressor bases in putrefying placenta, such as p-hydroxyphenylethylamine and (probably) isoamylamine.

In order to study the sympathomimetic ergone and its relation to nervous structures EULER (1946, c, d) made an extensive investigation of the action of different nerve tissue extracts. He found that extracts from the sympathetic trunk produced a large pressor action, sometimes corresponding to as much as 30—100 μg adrenaline per g nerve tissue (blood pressure test) whereas extracts from vagus nerve, sciatic nerve, spinal cord, dorsal and ventral roots and brain were rather poor in pressor activity. It was found that the thoracic and lumbar sympathetic chains and the splenic periarterial nerves were especially suitable for preparation of the active substance.

As "sympathetic indicators" EULER used the blood pressure of the cat in chloralose anaesthesia, the isolated non-pregnant and pregnant cat's and rabbit's uterus, the isolated rabbit's intestine and the cat's pupil. The stability of the sympathomimetic substance in the purified extracts was tested as well as its chemical

catechol and fluorescence reactions. All these indicators react for the active substance in the extracts in the same way as for nor-adrenaline, and EULER states: "The sum of evidence ... allows the conclusion that the sympathomimetic compound found in extracts of mammalian adrenergic fibres is different from adrenaline. On the other hand the active principle has the properties of a catechol compound, and a detailed study discloses a near relationship with a nor-compound. The assumption that the active substance is in fact the nearest relative to adrenaline, nor-adrenaline or catechol-ethanolamine, is suggestive."

From this investigation it became evident that the active substance in these extracts is "the physiological transmitter of adrenergic nerve action in mammals and identical with nor-adrenaline". It is of great biological importance that the sympathomimetic substance is also present in fairly large amounts in the adrenergic nerve trunks and its presence is obviously not restricted only to the nerve endings. This means that the substance in these extracts is not identical with "sympathin" since this conception refers to a combination product between the mediator released at nerve endings and a specific substance in the effector cells. In order to avoid confusion EULER suggests that the name "sympathin" should be used for the ergone demonstrated in adrenergic nerves, exerting the action of nor-adrenaline.

It is of further interest to note that the content of sympathomimetic substance in splenic extracts is considerably reduced after previous degeneration of the main portion of the periarterial splenic nerves.

EULER and SCHMITERLÖW (1947) showed that human and bovine blood contains a sympathomimetic substance which is obviously not adrenaline but acts more like nor-adrenaline.

The work of EULER was confirmed by BACQ and FISCHER (1947). They studied the effects of extracts, made according to EULER, from spleen, prostate, sympathetic chain, splenic nerves, stellate ganglion, coronary arteries and nerves and heart muscle (ventricular) obtained from various species. The extracts were tested on cat's blood pressure, denervated nictitating membrane and non-

pregnant uterus in situ. In splenic extracts they found a high eontent of a pressor substance obviously identical with nor-adrenaline. They also found a substance having the properties of nor-adrenaline in extracts from the sympathetie ehain, splenic nerves and stellate ganglion, but they ealled attention to the faet that it might be a mixture of adrenaline and nor-adrenaline at least in some cases, although the amount of adrenaline was considerably less than the amount of nor-adrenaline. In extracts of human eoronary arteries and nerves only adrenaline-effect was found or sometimes no effeet at all. For several reasons BACQ and FISCHER found it highly probable that the sympathomimetie substanee is really nor-adrenaline and they also demonstrate that the presenee of this substanee in the extraets eould not be looked upon as an artifaet. They suggest the possibility that in eertain tissues the synthesis of cateehol-amines is stopped at the stage of nor-adrenaline whereas in other tissues this nor-adrenaline is methylated to adrenaline.

In experiments on »liver sympathin» released on stimulation of the hepatie nerves, GADDUM and GOODWIN (1947) found that their results were compatible with the theory that liver sympathin is nor-adrenaline or perhaps tyramine. This latter suggestion does not seem very probable since the action of this amine is abolished by eocaine (BURN and TAINTER, 1931) whereas the effect of sympathin is enhanced by coeaine.

Referring to the works of EULER here eited, WEST (1947) made quantitative studies of adrenaline and nor-adrenaline. Using 13 different biologieal tests he determined the ratio of dose of dl-nor-adrenaline to equi-aetive dose of l-adrenaline and found that dl-nor-adrenaline was a stronger pressor agent and had a more powerful motor effeet on the pregnant cat's isolated uterus than adrenaline, but with all other tests studied its action was weaker. WEST pointed out that, contrary to BARGER and DALE (1910), the inhibitory properties of nor-adrenaline were not always slight whieh was elueidated through experiments on ileum preparations. This inhibitory action of nor-adrenaline and of extraets from sple-

nic nerves was observed already by EULER (1946 d) who found that the isolated non-pregnant cat's uterus responded with a slight inhibition to these agents. WEST also made the remark that nor-adrenaline almost always had a longer latency period and a longer recovery time than adrenaline even if the action was excitatory.

MARAZZI and MARAZZI (1947) using the inhibitory effect on transmission at synapses in sympathetic ganglia as a measure (MARAZZI, 1939) have also studied the inhibitory effect of nor-adrenaline. They found that nor-adrenaline exerted a considerable inhibitory action (as seen from a reduction in the height of the postsynaptic potentials) which was about one-half the inhibitory power of equi-molar quantities of adrenaline. This finding led them to conclude that nor-adrenaline could not be identical with CANNON and ROSENBLUETH's sympathin E since this postulated substance was a *purely* excitatory substance without inhibitory action. As an alternative they suggested that sympathin E be regarded as a *predominantly* (and not purely) excitatory agent.

In order to clear up this beginning confusion, EULER (1948 b) made the following statement:

Nor-adrenaline present in the nerves themselves cannot be the sympathin E as it was defined by CANNON and ROSENBLUETH. The sympathin E in its original meaning has never been prepared, but EULER believes "that the actions described by CANNON and ROSENBLUETH and attributed to sympathin E are due to the substance shown to be present in adrenergic nerves, having the properties of nor-adrenaline". BACQ and FISCHER (1947) suggested that any sympathomimetic ergone liberated from adrenergic neurones should be termed sympathin and if so, EULER suggested that the two distinctions *sympathin N* and *sympathin A* should be used, these terms referring to the transmitter having the properties of nor-adrenaline and adrenaline, respectively.

FOLKOW, FROST and UVNÄS (1948) studied the chemical transmission of vasoconstrictor impulses to the hind limbs and the splanchnic region of the cat. They stated that it was not likely

that the transmitter at vasoconstrictor nerve endings in the cat was adrenaline; to comply with their observations the transmitter should lack vasodilator properties. From their observations nor-adrenaline fulfills this requirement and their results gave further support to the theory concerning nor-adrenaline as the actual adrenergic transmitter.

PART II

PRESSOR AND DEPRESSOR ACTIVITY OF BLOOD VESSEL EXTRACTS

Chapter III

Previous investigations

VINCENT and SHEEN (1903) made extracts from different organs and found that probably all animal tissues contain both a pressor and a depressor substance. The pressor substance could be extracted by saline solution at ordinary temperatures, the depressor was extracted by boiling saline solution, which obviously destroyed the pressor substance. Nothing was said about the possible nature of these substances and no extracts were made from blood vessel walls. MILLER and MILLER (1911) found that alcoholic extracts in the case of *e.g.* liver, kidney, spleen, prostate etc. evaporated and redissolved in salt solution were without effect on dog's blood pressure. Alcoholic extracts of all nervous tissue contained a depressor substance. They pointed out that depressor substances in organ extracts might mask the effect of a possibly-present pressor substance.

HEILNER (1916—1921) found that watery extracts from the aortic wall contained a substance which lowered high blood pressure and was thought to be of therapeutic value in the treatment of arteriosclerosis. COLLIP (1928) described the occurrence of a non-specific pressor principle derived from a variety of tissues. The action of this pressor principle, obtained through extraction with acid boiling water, was antagonized by cocaine. Only in the case of prostatic extracts was the pressor effect enhanced by cocaine which led him to state that it "resembles that of epinephrine very closely". It must be pointed out that the amount of this non-specific pressor principle present in different organs was not very high: in order to cause a blood pressure rise of about 100 mm Hg (dog) a quantity of extract corresponding to 300 g ox spleen had to be injected. Extracts from blood vessels were not made.

In 1931, SATO reported an investigation concerning the effect of extracts from the middle layer of cattle aorta. The extraction was carried out during a two hour period using a concentrated salt solution. The extracts were tested on rabbit's blood pressure. The injection of an extract quantity corresponding to 0.1 g tunica media caused a marked and prolonged fall in blood pressure, which persisted after atropinization, vagus section or ergotaminization. This active substance was water soluble, insoluble in alcohol, ether, chloroform and aceton. It was not dialyzable.

Some "indirect" experiments regarding the presence of a sympathomimetic substance in blood vessels were performed by CANNON and BACQ (1931), BACQ (1933), ROSENBLUETH and CANNON (1932) and BACQ and BROUHA (1932). It was found that stimulation of the lower abdominal sympathetic strands influenced the vascular and pilomotor muscles of the tail region, and that the mediator carried by the blood stream accelerated the heart, raised the blood pressure, contracted the nictitating membrane and augmented the glucose content of the blood. When the cervical sympathetic was stimulated the tone of the small intestine of the cat was decreased and contraction of the denervated retractor penis occurred. An attempt was also made (ROSENBLUETH and CANNON, 1932), to separate the effects of stimulating vascular and pilomotor muscles, and it was found that in both cases a contraction of the denervated nictitating membrane occurred. BACQ and BROUHA confirmed these experiments. BAETJER (1930) found that the Orbeli phenomenon occurred even after ergotoxine administration which prevents vasoconstriction but does not check the production of sympathin (NAVRATIL, 1927, CANNON and BACQ, 1931, CANNON and ROSENBLUETH, 1933). An improvement of the muscular contraction was also obtained when the contralateral sympathetic chain was excited. BREMER (1932) suggested that sympathin liberated by the smooth muscles of the blood vessels was responsible for the favorable action obtained by stimulating the sympathetic fibres to a fatigued muscle.

When stimulating the adrenergic nerves to the perfused ear of a rabbit GADDUM and KWIATKOWSKI (1938, 1939) found that a

substance was liberated into the perfusion fluid which gave a specific colour reaction characteristic of adrenaline but not given by substances such as nor-adrenaline. It was also found (GADDUM, JANG and KWIATKOWSKI, 1939) that this substance inhibited the fowl's rectal caecum.

The paper by CANNON and LISSÁK (1939), previously cited, also dealt with the sympathomimetic properties in extracts from arteries (femoral, brachial, cervical and mesenteric) and liver vessels of the cat. They found that liver vessels which were freed from liver pulp as carefully as possible contained an active principle which exerted the following actions on sympathetic indicators:

1. A rise in blood pressure of the cat (pithed through orbit to midthorax or under dial anaesthesia). This blood pressure rise was enhanced by cocaine and reversed by ergotoxine. It must be pointed out, however, that in three instances the rise was preceded by an initial short fall — only in one instance was there a pure rise. ("Mixed and anomalous action of the extracts are attributed to peculiar dialysates from the tissues mentioned, that act oppositely to adrenaline").

2. An extreme pupillar dilatation (cat's iris sensitized by denervation and cocaine).

3. A contraction of the denervated nictitating membrane.

4. A *contraction* of the non-pregnant uterus — "not only had no relaxing effect... but actually a produced contraction". (For explanation, see paranthesis under 1.).

5. A positive chronotropic and inotropic effect on the hypodynamic frog heart.

From these facts they inferred that the substance was identical with adrenaline.

As to the interpretation of the above-mentioned findings some criticism may be advanced and this will be done in detail in the Discussion (Chapter VIII, page 97).

If the hepatic nerves were cut and allowed to degenerate the extracts of these sympathectomized liver vessels caused a pure

fall of pressure and had no effect on the iris or on the nictitating membrane.

The liver pulp did not exert any pressor action.

Although it was mentioned in this investigation that extracts were prepared from cat's femoral, brachial, cervical, and mesenteric arteries, little was said about their action. It was stated, however, that they relaxed the non-pregnant uterus and in the "Summary" it was said that extracts of organs containing adrenergic fibres act like adrenaline. It is difficult to judge from the text if this conclusion also is valid for extracts from the above-mentioned arteries.

In extracts from aorta and renal arteries RAAB (1943 a, b) investigated the presence and amount of substances giving the colour reaction described by SHAW (1938). He admitted that the results obtained with this method for the assay of adrenaline were due to the presence not only of adrenaline itself, but also of adrenaline-like substances containing a catechol nucleus and of ascorbic acid. In the human aorta, RAAB (1943 a) found a "colour unit" corresponding to 0.5 μg adrenaline per g tissue, the figure for human renal artery being 0.26. The "denominator of the specific ratio" (*i. e.* the ratio of the results with acid and with alkali, cf. SHAW) for these two tissue-extracts was high, which according to RAAB indicated pure or almost pure adrenaline *or sympathin.* This statement may be discussed since it depends on what is meant by sympathin. If sympathin is nor-adrenaline the statement must be revised, since it was pointed out by SHAW that treatment with alkali increases the colour produced by adrenaline, whereas such catechol compounds as nor-adrenaline showed no increase. If sympathin is nor-adrenaline then the high figures for the colour increase ratio (the "d. s. r.") given by RAAB would mean that aorta and renal arteries of man contain adrenaline. In an examination of the content of colour producing substances in human aorta from different ages and different degrees of arteriosclerosis, RAAB (1943 b) found values corresponding to from 0.17 to 0.62 μg adrenaline per g tissue, the lowest value was obtained from the age group 0—7 years, the highest from people

suffering from high blood pressure. In renal arteries no significant difference in the average concentration of the total chromogenic material was found between sclerotic and non-sclerotic arteries. The values were generally lower than those for aorta.

Using the extraction and purification method described by EULER (1946 d), BACQ and FISCHER (1947) found that human coronary arteries contained only adrenaline and no traces of a sympathomimetic substance with nor-adrenaline-like action.

In a preliminary investigation SCHMITERLÖW (1948) found that extracts (prepared according to the method described by EULER, 1946 d) from the aortic wall of cattle contained a depressor and a pressor substance, the depressor substance obviously to a large extent consisting of histamine and the pressor substance behaving like nor-adrenaline.

Chapter IV

Methods

A. Extraction procedures

1. The extraction procedure was as a rule that described by EULER (1946, a, b, d).

The material used was collected at the slaughter-house as soon as possible after the animals (cattle, horse, pig) were killed or from newly killed experimental animals (dog). The blood vessels used were freed as carefully as possible from connective tissue. The material was minced in an electric mill or ground with sea sand (smaller vessels) and then extracted for two hours with two volumes of acid alcohol (2 ml 10 N H_2SO_4 per kilogram fresh tissue). After filtering on a Buchner funnel and washing of the precipitate with acidified alcohol the filtrate was evaporated in vacuo to a small volume. The fatty materials in these extracts were removed with ether in a separatory funnel. The ether was poured off and any remaining traces of ether were evaporated in vacuo. The pH of the extracts was about 4.5 throughout this preparatory procedure.

2. Sometimes the extraction was carried out in a somewhat different way. The minced material was then extracted with Ringer's solution to which was added a small amount of tricresol in order to prevent putrefaction. The extraction was continued for about 6 hours at 37° C. The Ringer's solution was then filtered off and 2 volumes of acid alcohol were added to the filtrate. After precipitation for 12 hours the precipitate was filtered off and the clear filtrate evaporated in vacuo to a small volume, the lipids being subsequently removed with ether.

B. Purification of the extracts

When tested on cat's blood pressure the extracts made according to the above-mentioned procedures often gave a pronounced initial lowering of the blood pressure followed by a secondary rise. It was found by EULER (1946 d) that the easiest way of removing this depressor phase was by treating the "erude" extraets with fullers' earth at a slightly acid reaction. This clay adsorbs the concomitant depressor substances leaving the pressor phase fairly intact (although some loss of pressor activity is regularly observed — more or less depending on the fullers' earth used).

The "fullers' earth" used in this investigation has been manufactured by the The Fullers' Earth Union Ltd, Surrey, England. Fullers' earth is an adsorbent clay composed mainly of a clay mineral called "montmorillonite" containing aluminium, silicon, magnesium, iron, oxygen and hydroxyl groups. The formula $(Al, Fe, Mg)_4$, Si_8, O_{20}, $(OH)_4$ represents the main structure but to this must be added the exchangeable ions (usually Ca, Mg, Na and H in varying quantities) and the water content. The adsorptive capacity of fullers' earth for basic organic compounds is due to its base exchange capacity.

Several different types of "activated" fullers' earth have been tested, the most suitable one being "fullers' earth K N 11 C". Other fullers' earth, such as "Fulmont 500", "Fulmont 700" and "Fulbent" could not be regarded as suitable since they adsorb, to a large extent, not only the "depressor phase" but also the "pressor phase". Also in adsorption experiments with pure adrenaline or l-nor-adrenaline in watery solutions these latter earths were found to yield unsatisfactory results.

C. Histological examination

The histological examination of the blood vessels was carried out in the Department of Anatomy and Histology (Head: Professor A. PALMGREN), Kungl. Veterinärhögskolan, Stockholm. The nerve staining method used was that described by PALMGREN (1948).

D. Biological tests

1. Blood pressure

Cats were used as test animals throughout this investigation. They were anaesthetized with chloralose — 7 ml of an 1 % solution in physiological NaCl per kg intramuscularly. In order to render the blood pressure preparation more sensitive 8 mg/kg cocaine hydrochloride i. m. was sometimes given. In most cases both adrenals were completely removed, which will be stated in the text to the figures.

The blood pressure was recorded from the carotid artery. All injections were made through a cannula inserted in the femoral vein. When different solutions were to be tested against each other it was thought advisable to adjust the concentrations to give approximately the same amount of liquid injected so as to obtain equi-pressor results. The rate of injection was kept fairly constant and the "dead space" of the cannula was very small in order to avoid the use of large amounts of Ringer's solution when washing out the cannula. Between the injections a small amount (0.5 ml) of Ringer's solution was injected through the cannula in order to prevent admixture from the preceding injection.

In order to reverse the pressor effect of adrenaline 0.5 mg dihydroergotamine methansulfonate or 15 mg dibenamine per kg of body-weight were given intravenously.

2. Uterus in situ

When testing the blood pressure of female cats the movements of the uterus were also recorded on the kymograph. A small incision was made in the abdominal wall and the lower portion of the uterine body lightly fixed to the wall. The movements of the uterine body were transmitted to a recording lever. Pregnant as well as non-pregnant cats were used.

3. Heart rate

The heart rate of the cats was observed either directly on the blood pressure traeing (the movements of the mercurial column) or in the following way. A deviee in which the R-spikes of the ECG were amplified gave impulses to a relay which for each impulse emitted a sharp sound. The frequeney of the heart could thus be overheard at the same time as the effeet on the blood pressure was watched.

4. Isolated guinea-pig's intestine

The animals were starved for about 12—15 hours before the experiment as this seemed to render the intestine more sensitive. They were then killed by a blow on the head and the blood vessels of the neck were opened. When the animals had finished bleeding a portion of about 30—40 cm in length of the small intestine was removed and placed in luke-warm Tyrode's solution. After about 20 minutes the intestine showed strong spontaneous movements and a piece of it was fixed in the intestinal bath, aerated by a mixture of 95 % O_2 and 5 % CO_2 ("Karbogen"). The size of the intestinal bath was 20 ml.

The composition of the Tyrode's solution which was used as suspension fluid throughout all the experiments was as follows: 0.8 % NaCl, 0.02 % KCl, 0.02 % $CaCl_2$, 0.02 % $MgCl_2 \cdot 6\,H_2O$, 0.1 % $NaHCO_3$, 0.005 % $NaH_2PO_4 \cdot H_2O$ and 0.1 % glucose.

E. Physical test

The fluoreseence test deseribed by GADDUM and SCHILD (1934) was performed on the different extraets. In order to obtain an optimal fluoreseence the amount of alkali added was ealculated so as to give a pH of approximately 12; aecording to the figures given by JÖRGENSEN (1945, 1948) who for 2 ml of pure adrenaline

solution added 0.3 ml 1 N NaOH and to blood dialysates 0.3 ml 2 N NaOH.

The fluorescence determinations were carried out in a Lumetron fluorescence meter, the degree of fluorescence being observed one minute after the addition of alkali.

F. Chemical test

The colour reaction with arsenomolybdic acid after the adsorption of the colour-producing substances (adrenaline, nor-adrenaline or the sympathomimetic substance of the extracts) on aluminium hydroxide was used according to the original method described by SHAW (1938). The *amount* of sympathomimetic substance was not determined, only the difference in colour intensity when performing the reaction in alkaline or acid medium. If adrenaline is present the colour is definitely increased when alkali is added, if *e. g.* nor-adrenaline is present there is no difference in colour.

The colour determinations were carried out in a Lumetron colorimeter.

G. Substances used

Acetylcholine chloride — F. Hoffman—LaRoche & Co. A. G., Basel. Fresh solutions were made for each experiment.

Acetyl-β-methylcholine chloride — Neo Chemical Corp., New York.

Adenylic pyrophosphate — C. H. Boehringer Sohn, Ingelheim am Rhein.

Adrenaline hydrochloride — the commercial 1 $^0/_{00}$ l-adrenaline hydrochloride solution from Parke, Davis & Co. Dilutions were made in distilled water and the pH adjusted to about 4.5.

dl-nor-Adrenaline hydrochloride — samples were kindly put at my disposal by Professor U. S. von Euler, Physiological Dept., Karolinska Institutet, and were later obtained from Winthrop-Stearns Inc., New York. Solutions were made in distilled water and the pH adjusted to about 4.5.

l-nor-Adrenaline hydrochloride — Winthrop—Stearns Inc., New York. Solutions — see above.

Atropine sulfate — commercial samples.

L-Chloralose — F. Hoffman—LaRoche & Co. A. G., Basel.

Choline chloride — Schering—Kahlbaum A. G., Berlin.

Cocaine hydrochloride — commercial samples.

Dibenamine (N, N-dibenzyl-chloroethylamine) — made for experimental purpose by Mr. S. Carlsson, A. B. Recip, Stockholm.

Dihydroergotamine methansulfonate — kindly put at my disposal by Messrs. Sandoz A. G., Basel.

Histamine dihydrochloride — F. Hoffman—LaRoche & Co. A. G., Basel. Fresh solutions were made for each experiment.

Lergitin (dimethylaminoethylbenzylaniline) — the pure substance was put at my disposal from A. B. Recip, Stockholm.

Nicotine hydrochloride — Th. Schucardt, GmbH, Görlitz.

Pilocarpine hydrochloride — commercial samples.

Substance P (EULER and GADDUM) — a preparation of this substance was kindly put at my disposal by Professor U. S. von Euler.

The amounts of the substances are always expressed as salts.

When comparing the blood pressure effect of the l-adrenaline, dl-nor-adrenaline and l-nor-adrenaline used in this investigation and produced by the above-mentioned manufacturers the following order of activity and correlation figures were *usually* obtained (adrenalectomized cats):

l-adrenaline	1
dl-nor-adrenaline	1.2
l-nor-adrenaline	1.8

These values are calculated from doses within the range of 0.125—1 μg per kg body-weight of the three substances tested.

Chapter V

Experimental tests of methods

A. Extraction procedures

In order to see if the extraction procedure described by EULER (1946) caused any marked loss of activity of the possibly present sympathomimetic substance, the following experiments were performed.

1. Watery solutions of l-adrenaline, dl-nor-adrenaline and l-nor-adrenaline were extracted according to EULER's method. When comparing the pressor effects of the original solutions with those of the same extracted solutions no difference could be observed (Fig. 1). It could also be stated that this extraction proccdure did not change the nature of these three compounds as judged from the effect on the non-pregnant uterus in situ, the extracted adrenaline still giving a pronounced relaxation, the other two showing no influence on the tonus and rhythm of the uterus.

In this "test" experiment no organic tissue was present as was the case with extracts of blood vessel walls.

2. For that reason the same extraction procedure was carried out with three portions of minced human placenta to which were added l-adrenaline, dl-nor-adrenaline and l-nor-adrenaline respectively in known concentrations. As has been shown by EULER (1945) extracts from the human placenta did not contain any sympathomimetic agent and the placenta was thus suitable for this purpose for two reasons: 1. there was no contamination with pressor substance from the tissue itself and 2. there was an abundance of blood vessels presumably containing the same sort of organic materia as other blood vessel walls.

The results from these "test" experiments can be seen in Fig. 2.

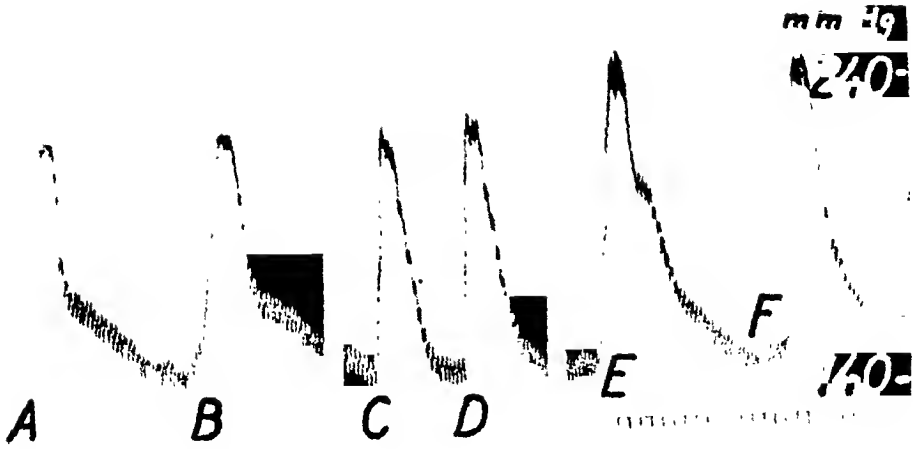

Fig. 1. Cat, blood pressure. Chloralose i. m.

A. 5 μg adrenaline.
B. 5 μg adrenaline, extracted.
C. 5 μg dl-nor-adrenaline.
D. 5 μg dl-nor-adrenaline, extracted.
E. 5 μg l-nor-adrenaline.
F. 5 μg l-nor-adrenaline, extracted.

Time 10 secs.

The extract of human placenta (without the addition of sympathomimetic substances) gave a sharp fall in blood pressure but no secondary rise (adrenalectomized cats). After a previous administration of atropine and Lergitin (antihistamine drug) this depressor effect was abolished and the effect of the extract was practically nil (Fig. 2 D).

It could be observed that there was a slight loss in the activity of the sympathomimetic substances in the extracts made with human placenta as organic materia. This loss was, however, not great and it was not thought worth while to perform a series of experiments in order to obtain a statistical value, since such a figure would reveal nothing. It seems sufficient to state that the loss was slight (probably not more than the loss occurring when shaking the extracts with ether, cf. EULER, 1946 d). This is of course no proof that all of the prevailing sympathomimetic substance in blood vessel walls was really extracted. Some of it might be present in a bound, unextractable form (cf. EULER, 1946 d) or the disaggregation of the material might be incomplete, thus preventing the extraction liquid from coming in close contact with the tissues.

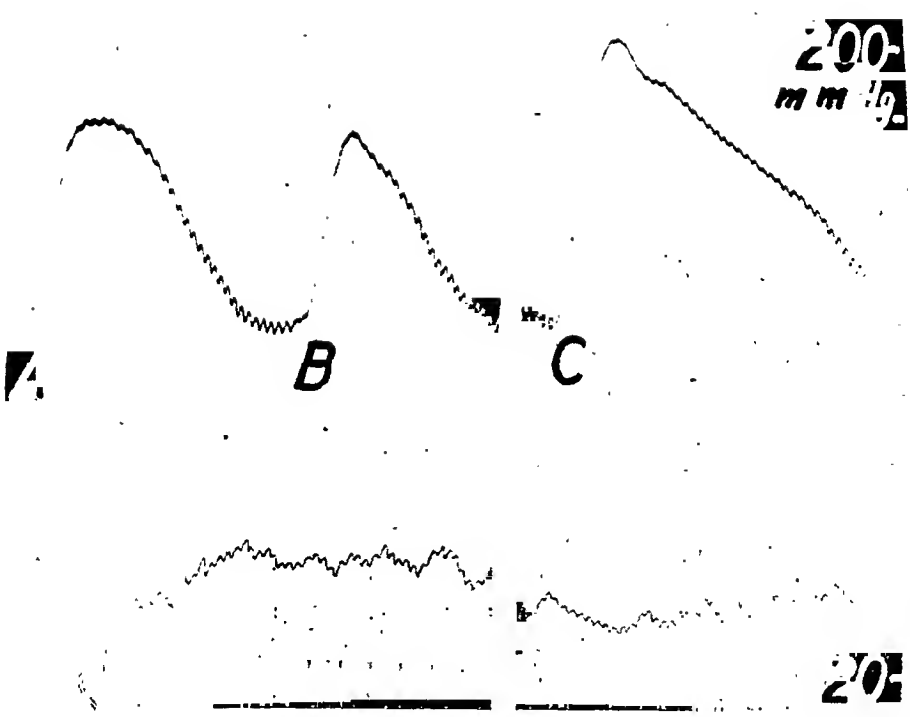

Fig. 2 a. Cat, blood pressure (upper curve), non-pregnant uterus in situ (lower curve). Both adrenals removed. Chloralose i. m.
After previous administration of 2 mg/kg Lergitin and 0.25 mg/kg atropine i. v.
A. 1 μg adrenaline.
B. 1 μg dl-nor-adrenaline.
C. 1 μg l-nor-adrenaline.
Time 10 secs.

It was also of interest to note that in the presence of organic tissue there was no change in the mode of action of the three tested compounds (see uterus-curve, Fig. 2).

3. If the whole procedure of extraction, evaporation and ether shaking was carried out under nitrogen gas, no higher yield of pressor substance was obtained (experiments with cattle aorta). It was thus obvious that if there occurred any oxidation of the pressor substance during the course of ordinary extraction at a pH of about 4.5 it was very slight.

4. In the extraction method described by EULER (1946 a) the time of extraction was stated to 1—2 hours. If the extraction

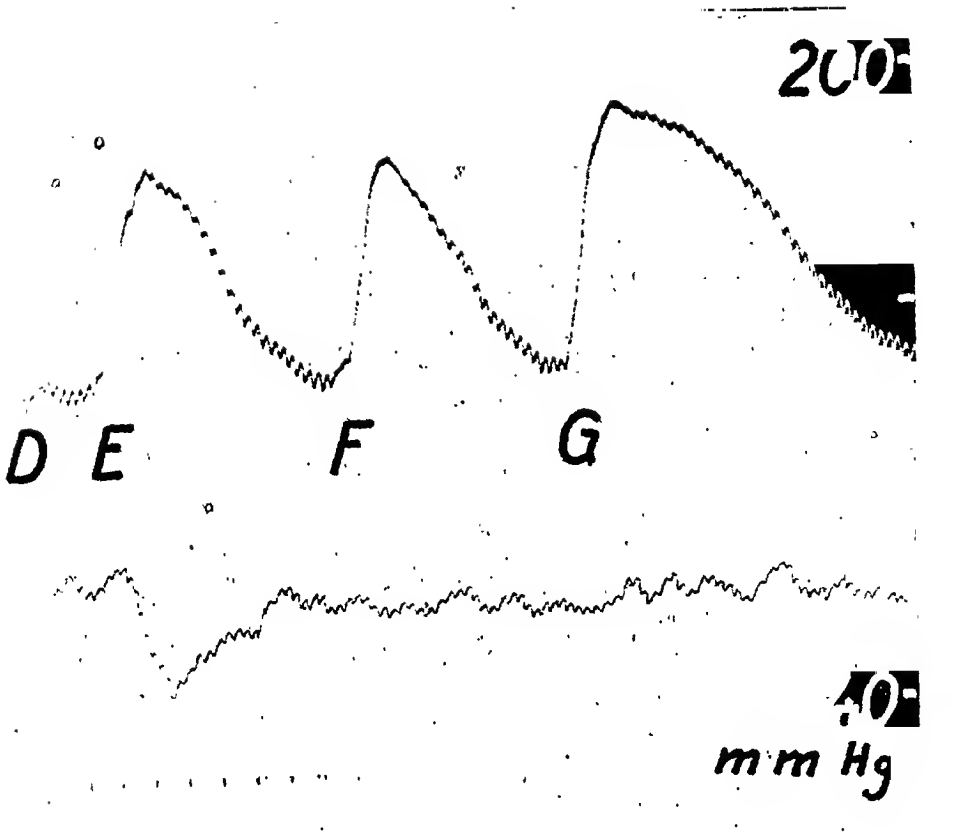

Fig. 2 b. Same cat as in Fig. 2 a.

D. Extract from placenta, corresponding to 1 g placenta.
E. Extract from placenta with addition of 1 μg adrenaline per g tissue. Injected volume corresponding to 1 g placenta and 1 μg adrenaline.
F. Extract from placenta with addition of 1 μg dl-nor-adrenaline per g tissue. Injected volume corresponding to 1 g placenta and 1 μg dl-nor-adrenaline.
G. Extract from placenta with addition of 1 μg l-nor-adrenaline per g tissue. Injected volume corresponding to 1 g placenta and 1 μg l-nor-adrenaline.

time was prolonged no higher yield of active substance was obtained — on the contrary, a certain loss of activity was observed after extraction for 5 hours (Fig. 3).

If the collected material was not extracted at once but allowed to stand in open air at room temperature the amount of pressor substance gradually decreased. The extract of cattle aorta which had been kept in the minced state for 2 days did not show any significant pressor action on the blood pressure.

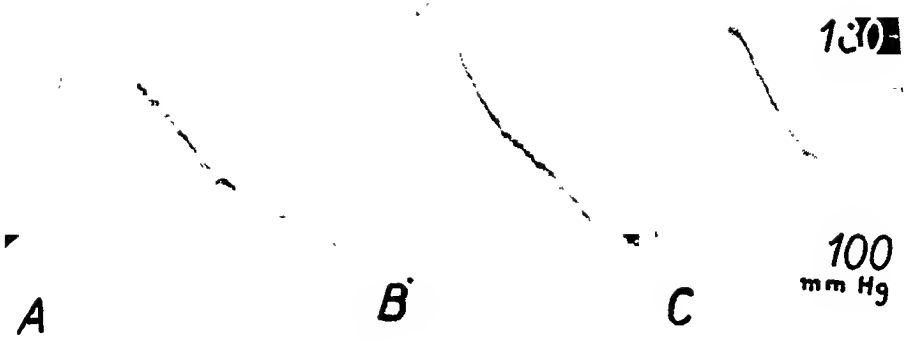

Fig. 3. Cat, blood pressure, chloralose i. m.

After previous administration of cocaine, Lergitin and atropine.

A. Aortic extract corresponding to 2 g cattle aorta, treated twice with fullers earth (5 mg per ml extract). Extraction time 1 hour.

B. Aortic extract (as above). Extraction time 3 hours.

C. Aortic extract (as above). Extraction time 5 hours.

Time 10 secs.

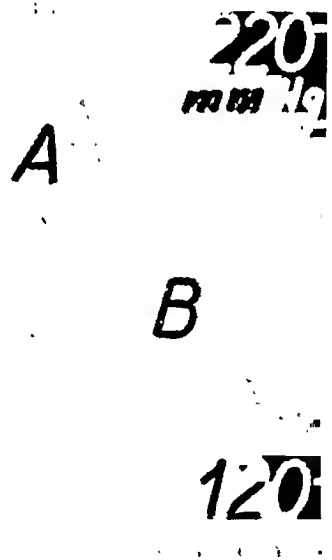

Fig. 4. Cat, blood pressure. Both adrenals removed. Chloralose i. m.

A. Aortic extract corresponding to 2 g horse aorta, extraction according to *Euler's* method.

B. Aortic extract corresponding to 2 g horse aorta, extracted with tricresol-Ringer.

Time 10 secs.

If the material was not collected at once when the animal was killed but after a lapse of 1—2 hours no significant decrease of activity could be noticed (dog's aorta). If a longer time was allowed to pass there was a distinct decrease in the activity of the extracts.

5. The other method of extraction (with tricresol-Ringer for 6 hours at 37° C) also gave extracts containing a pressor (and depressor) principle. This method was, however, abandoned, since the yield was considerably less (Fig. 4). Even if the extraction time was only 2—3 hours there was no higher yield.

6. The extracts were not stable even when kept in the refrigerator at 4° C and at pH 4.5. After 1—2 weeks the pressor activity was somewhat diminished and it thus seemed advisable to perform the tests as soon as possible.

B. Purification procedure

As has been pointed out by EULER (1946 d) and SCHMITERLÖW (1948) the adsorption procedure with fullers' earth should be carried out at a slightly acid reaction in order to remove the depressor phase of the organ extracts. From a previous investigation (SCHMITERLÖW, 1948) it was obvious that at least some part of the depressor activity in extracts from aortic walls was due to the presence of histamine. Another part is probably due to the presence of acetylcholine (see Chapter VI). It was then thought worth while to determine the optimum adsorption pH for these two substances.

a. Histamine. Histamine dihydrochloride solutions (10 μg per ml) were treated with fullers' earth (KN 11 C) in a concentration of 5 mg per ml of this histamine solution at different pH. After the addition of the fullers' earth the pH was immediately adjusted to the desired value and the mixture was slowly shaken 50 times. The mixture was then centrifuged and fullers' earth was again added to the clear supernatant liquid, the whole procedure was thus repeated. After this second treatment the pH of the different

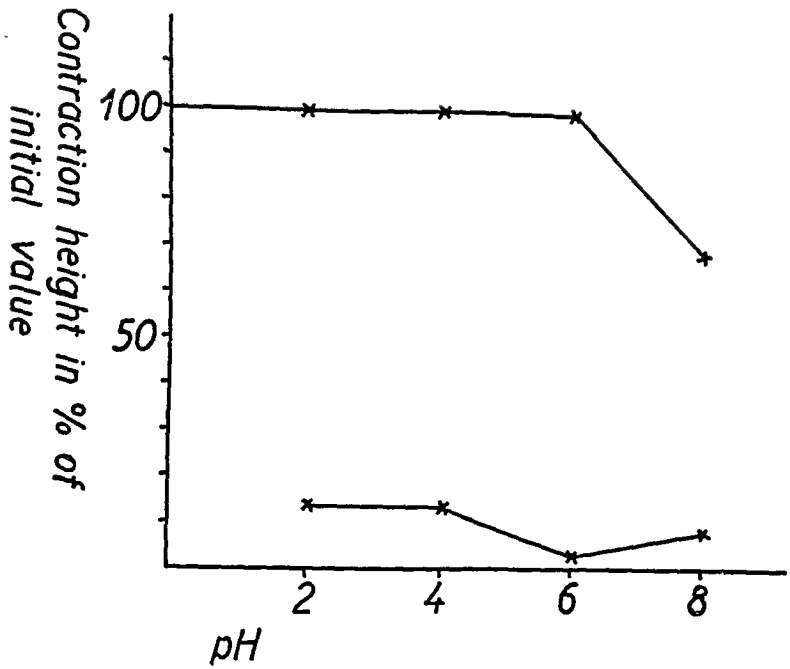

Fig. 5. Upper line: Influence of different pH on histamine activity.
Lower line: Remaining histamine activity (in % of initial contraction height) after adsorption to fullers' earth at different pH.

solutions was adjusted to pH 6 and the histamine effect was tested on isolated guinea-pig's intestine. The effect of the untreated histamine solution was placed at 100 and the action of the fuller-treated histamine solutions was then calculated in percent of this effect. The result can be seen in Fig. 5.

In order to determine if the reduced histamine activity really was due to an adsorption to the fullers' earth and not only to changes in pH the same procedure was carried out but without the addition of fullers' earth, *i. e.* exposure of histamine solutions to different pH. No significant change in activity could be observed until the pH reached 8, when the histamine activity was reduced to about 70 % of the original value in about 30 min.

The results showed that the optimum adsorption of histamine to fullers' earth took place at a pH of about 5—6, that is, at a pH which would not in itself destroy adrenaline or nor-adrenaline.

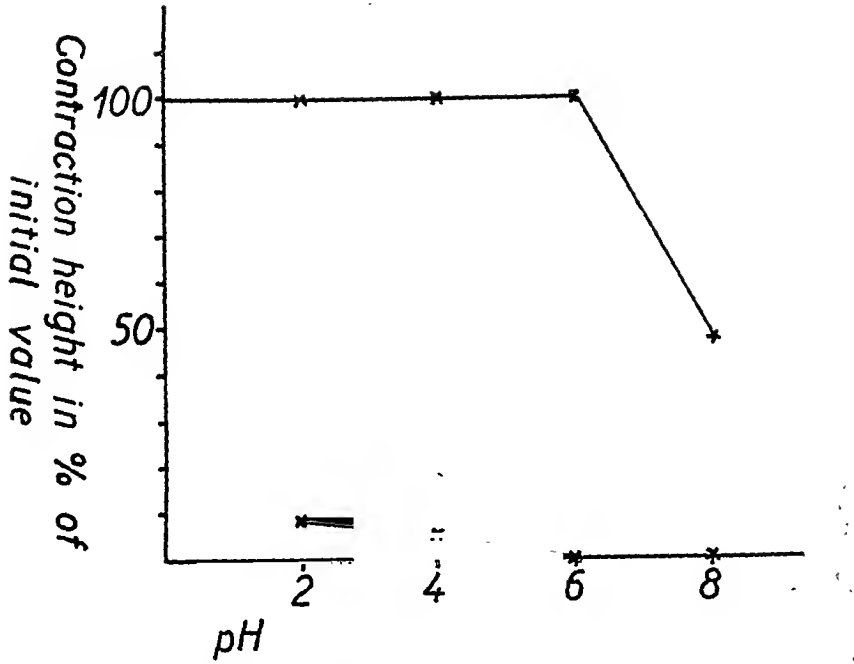

Fig. 6. Upper line: Influence of different pH on acetylcholine activity.
Lower line: Remaining acetylcholine activity (in % of initial contraction height) after adsorption to fullers' earth at different pH.

b. Acetylcholine. The same procedure was carried out with acetylcholine in order to determine if the adsorption of this substance followed the same course as that of histamine. This was obviously the case, the maximum adsorption beginning at a pH of about 5. Fig. 6.

The treatment with fullers' earth also caused a loss in activity of the sympathomimetic substances. This error could be corrected if the pressor effect of the extracts was always compared with fuller-treated standard solutions of adrenaline or nor-adrenaline.

It may be mentioned that if samples of a solution of *e. g.* dl-nor-adrenaline were treated twice with increasing amounts of fullers' earth (KN 11 C) the loss of activity did not follow a straight line. The diagram in Fig. 7 given below shows the correlation between loss of activity (blood pressure test) and amount of fullers' earth used for the adsorption.

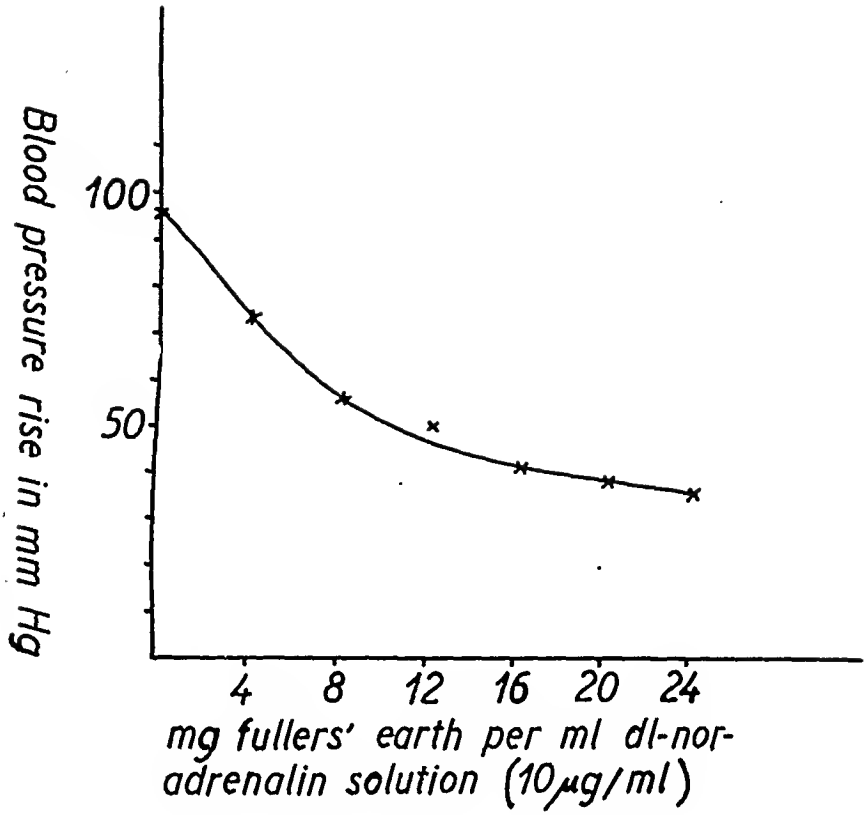

Fig. 7. Pressor activity of dl-nor-adrenaline after treatment with different amounts of fullers' earth.

C. The fluorescence test.

When testing the fluorescence of the extracts it was found that they had a bluish fluorescence colour even before the alkali was added. This fluorescence of the extracts themselves could be largely reduced by treating the extracts with aluminium hydroxide at pH 4. With this treatment some fluorescence giving substances of the extracts were obviously adsorbed to the aluminium hydroxide, whereas in "test" experiments no adsorption of adrenaline or nor-adrenaline as judged from both fluorescence and blood pressure tests, could be observed to take place at this pH.

The aluminium hydroxide used was precipitated according to the method described by SHAW (1938).

D. Some remarks on the biological test methods

When BARGER and DALE (1910) introdueed the term "sympathomimetic" they emphasized that the production of a rise of arterial blood pressure was not in itself sufficient to elassify an aetion as sympathomimetic. The blood pressure rise affords a eonvenient quantitative index in this respeet but really some or all of the other effeets of sympathetic nerves would have to be observed. They pointed out that the cat's uterus is partieularly valuable as an index since this organ is only innervated by sympathetie nerves. Its response to sympathetic nerve stimuli and to adrenaline is, in the main, motor during pregnancy and inhibitor during the non-pregnant state. "We believe", BARGER and DALE wrote, "that the assoeiation of a pressor aetion, due to arterial constriction and cardio-acceleration, when the substance is injected into the whole animal, with inhibition of the tone and rhythm of the isolated nonpregnant uterus of the cat, is almost sufficient evidence to warrant the attribution to a substance of an action of the sympathomimetic type".

In the prcsent investigation the blood prcssure of the cat has been simultaneously recorded with the movements of the uterus in situ. It seemed advantageous to use the uterus in situ, since the effect of the injected substanees and extracts eould then be observcd on blood pressure and uterus at the same time in the same animal and using the same (or equi-pressor) doses. In the non-pregnant state the eat's uterus in situ responded to l-adrenaline with a marked relaxation whereas dl-nor-adrenaline and l-nor-adrenaline in the main did not eause any effeet. Sometimes a very slight relaxation was observed when giving large doses of l-nor-adrenaline but this was in faet an exception. In the pregnant state the cat's uterus responded to both adrenaline and nor-adrenaline with an inereased tone. There was often, however, a differenee in the type of response, the effect of nor-adrenaline being more prolonged. After the contraction phase whieh was equally sharp for adrenaline and for nor-adrenaline, the uterus relaxed rather quickly after adrenaline and resumed its original

tone whereas after nor-adrenaline the relaxation comes on more gradually (see Fig. 22, page 81).

The cardio-accelerator effect of the substances or extracts injected were regularly observed by watching the pulse frequency on the blood pressure tracing or through listening to the heart frequency by means of the device described in Chapter IV.

The nictitating membrane has been used by many authors as a "sympathetic indicator". Its response to adrenaline and nor-adrenaline is excitatory for both, adrenaline being somewhat more active (WEST, 1947). The nictitating membrane is extremely suitable for testing sympathomimetic substances but it will not give any information about the question "adrenaline or nor-adrenaline?".

The cat's pupil has also been widely used. Adrenaline and nor-adrenaline both give a dilatation of the pupil, the former, however, being more potent (EULER, 1946 d). Also, in this case, the ratio of dose of nor-adrenaline to equi-active dose of adrenaline was not as high as in the case of the non-pregnant uterus of the cat.

The isolated rabbit's intestine also offers a good "sympathetic indicator". In some preliminary experiments, however, it was found rather difficult to distinguish with certainty between adrenaline and nor-adrenaline (Fig. 8), although it is stated that the former exerts a more pronounced inhibitory effect. Even when using equi-pressor amounts of the three substances l-adrenaline, dl-nor-adrenaline and l-nor-adrenaline no difference in effect could be observed in some cases.

It was previously pointed out by BARGER and DALE (1910) that ergotoxine in sufficient doses reversed the pressor effect of adrenaline whereas the effect of nor-adrenaline was certainly diminished but not reversed. It was shown by, amongst others, CANNON and ROSENBLUETH (1933) that the blood pressure raising effect of sympathin, released by sympathetic stimulation, was not reversed by ergotoxine. EULER (1946) showed that ergotamine did not reverse the effect of the sympathomimetic substance in extracts from spleen and adrenergic nerves. The use of ergotamine

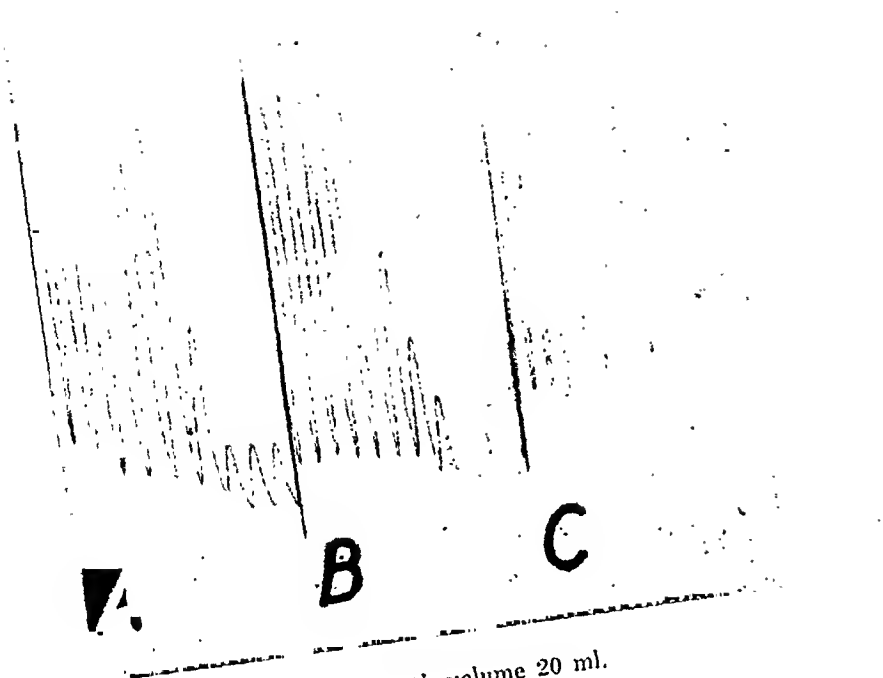

Fig. 8. Isolated rabbit's intestine. Bath volume 20 ml.
A. 1 µg adrenaline.
B. 1 µg dl-nor-adrenaline.
C. 1 µg l-nor-adrenaline.

(or dihydroergotamine which is still more active as a sympathicolytic agent) seems to offer a suitable method of differing adrenaline from nor-adrenaline. The same holds true also for some other substances, such as the Fourneau compound F 933 (piperidino-methylbenzodioxane) and dibenamine (NICKERSON and GOODMAN, 1947).

Chapter VI

Pressor and depressor activity of extracts from aortic wall. Experimental results

I. Cattle aorta

A. Preliminary observations

The injection of unpurified extracts from aortic wall into the cat caused a pronounced initial fall of blood pressure followed by a secondary elevation and an acceleration of the heart. After two treatments with fullers' earth at pH 5 the depressor effect of the extracts usually disappeared leaving a pure pressor effect. This pressor effect was enhanced by cocaine and abolished after treatment of the extracts with iodine at pH 8.

After a dose of dihydroergotamine sufficient to reverse the pressor effect of adrenaline the effect of the purified extracts was in the main diminished but not reversed. It was, however, observed that in some cases the effect of the extracts was reversed.

On the non-pregnant cat's uterus in situ the purified extracts did not, as a rule, give any relaxation, the tone and rhythm of the organ being uninfluenced. It sometimes occurred, however, that the effect of the extracts was more like the action of adrenaline, causing a distinct relaxation of the uterus.

The above-mentioned irregularity in regard to the biological effects of the extracts seemed rather puzzling and as it could hardly be assumed that the sympathomimetic substance in some cases acted like nor-adrenaline and in some cases like adrenaline some other explanation was obviously needed.

It was then observed that one and the same extract had acted like nor-adrenaline in one of the test-animals and like adrenaline in another cat. It was furthermore observed that if the removal of the depressor phase was not complete, leaving a small but visible initial lowering of the blood pressure, the extracts from different aortas almost regularly exerted a distinct inhibitory action on the non-pregnant uterus. These findings, combined with the fact that these extracts according to results with SHAW's colour reaction did not contain adrenaline but some other colour producing substance, led the author to investigate closer the nature of the depressor phase.

B. The depressor activity

After adsorption to fullers' earth the depressor effect of the extracts had usually disappeared. The depressor substance (or substances) was then obviously adsorbed to the earth and it was tried to elute it from this. It was shown in a previous paper (SCHMITERLÖW, 1948) that pyridine could be used as an eluting liquid. The method was as follows: The crude aortic extracts were treated twice with fullers' earth at pH 5 and after centrifuging, both portions of the earth were collected. This earth was then treated for 30 minutes with a 10 % watery solution of pyridine at pH 8, the mixture being occasionally shaken. The fullers' earth was then filtered off and the filtrate evaporated in vacuo. The pH was then adjusted to neutral.

It has already been mentioned that the fullers' earth also adsorbs some of the pressor constituent of the extracts. From "test" experiments it could be shown, however, that if a solution of nor-adrenaline was treated with fullers' earth and this earth then exposed to the pyridine-solution at pH 8 for 30 minutes no traces of nor-adrenaline could be observed as judged from blood pressure test. It was thus hardly likely that any blood pressure raising constituent was present in these eluates.

When injecting these eluates in the cat there was a sharp fall in blood pressure followed by a more or less marked blood pressure

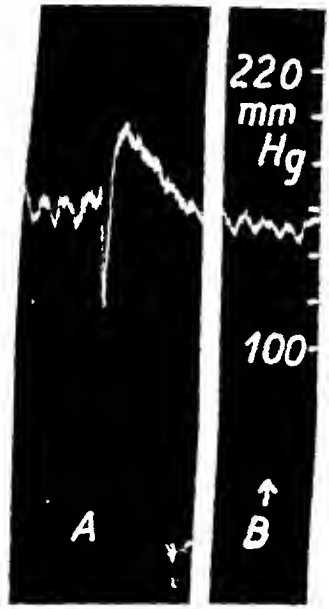

Fig. 9. Cat, blood pressure (upper curve), non-pregnant uterus in situ (lower curve). Adrenals intact. Chloralose i. m.

A. Eluate from fullers' earth, used for adsorption of depressor substances in aortic extract (the added amount corresponds to 3 g cattle aorta).

B. The same as A but after administration of 2 mg Lergitin per kg body-weight i. v.

rise and a definite relaxation (during the pressor phase — during the depressor phase there was often a slight increase in tone) of the non-pregnant uterus (Fig. 9). As it is well known that animal tissues contain histamine the effect of the antihistamine drug Lergitin was tested. After a previous administration of 2 mg Lergitin per kg body-weight intravenously the depressor effect of the eluates was very much diminished, no secondary rise and no relaxation of the uterus now being observed.

The eluates of the fullers' earth gave a strong contraction of the isolated guinea-pig's small intestine and this effect could be completely abolished after a previous addition of a small dose of Lergitin to the intestinal bath.

When testing the crude extracts (not treated with fullers' earth) of the aortic wall of cattle on the isolated guinea-pig's small intestine it was found that they gave very strong contractions of

the intestine. This effect could not, however, be totally abolished by small doses of Lergitin, a small contraction still occurring which was abolished after the addition of rather large amounts of the antihistamine drug or after small doses of atropine.

Determinations of the specific and non-specific action of Lergitin and atropine

The modern antihistamine drugs are extremely useful as a means of securing the histamine-identity of unknown depressor substances in various extracts. It must, however, always be kept in mind that the pharmacodynamic action of the antihistamine drugs is not only limited to a specific histamine antagonizing effect, they also exert a general spasmolytic action. When using the guinea-pig's isolated small intestine as a test organ for histamine the effect of this substance can be fully inhibited by the addition of small doses of an antihistamine drug. Adding larger amounts of antihistamine drug not only blocks the effect of histamine but also the action of other spasmogenic substances, such as acetylcholine, barium chloride, pilocarpine, K-ions, substance P, nicotine etc. It is thus necessary to determine the ratio between the specific dose — the amount of antihistamine drug which only blocks the effect of histamine — and the non-specific dose which inhibits the effect of all spasmogenic substances. Only under such precautions can the antihistamine drugs be used as a sort of "specific denominator" for histamine.

The antihistamine drug used throughout this investigation was N-phenyl-N-benzyl-N', N'-dimethylethylenediamine hydrochloride. This substance was originally described by HALPERN (1942) and was marketed under the name of "Antergan". In this research the corresponding Swedish drug "Lergitin" has been used. In order to determine the ratio between specific (histamine antagonizing) and non-specific (antispasmodic) dose a special series of experiments were performed.

Methods

1. Isolated small intestine of guinea-pig was used as test object (for experimental details see Chapter IV). The effect of Lergitin was tested on intestinal spasms induced by histamine, acetylcholine, barium chloride, choline, acetyl-β-methylcholine, pilocarpine, substance P (EULER and GADDUM), adenylic pyrophosphate, nicotine and potassium chloride. This was done in order to elucidate the specificity of antihistamine action of Lergitin.

The above-mentioned spasmogenic substances were given in doses sufficient to cause submaximal contractions and after three or four consecutive doses, giving fairly constant contraction heights, the antihistamine drug was added to the intestinal bath and without changing the suspension fluid the same amount as before of intestine-contracting substance was added. This procedure was repeated several times, beginning with small doses of antihistamine drug and finishing with a dose capable of completely blocking the effect of the succeeding spasmogenic substance. The average of the contraction heights of the preceding three or four doses was calculated and compared with the contraction height after the addition of a certain amount of antihistamine drug. The remaining contraction height was expressed in percent of the initial contraction height.

In order to make a graphical representation of the results the remaining contraction heights (in % of initial value), induced by the intestine-stimulating drugs, were plotted against the logarithmic value of the number of moles per ml of bath fluid of the antihistamine drug. The curves thus obtained showed the relation between the concentration of antihistamine drug and the blocking effect of this drug on the intestinal contractions caused by different spasmogenic substances. The curves were, as a rule, obtained as mean values from three or four experiments with intestinal pieces from different animals.

The same type of experiment was also carried out with atropine in order to study its antispasmodic effect on different spasmogenic substances.

2. Blood pressure. The ability of Lergitin to prevent the fall in blood pressure due to histamine was studied in cats. Here also this effect was compared with the action against a fall in blood pressure caused by other drugs, such as acetylcholine, choline, acetyl-β-methylcholine and substance P.

The histamine antagonizing and general spasmolytic effect of antihistamine drugs have been investigated by several authors (see LOEW's review, 1947). It is commonly stated that the different antihistamine drugs relax the histamine-induced spasm of the isolated intestine at smaller concentrations than are necessary to abolish the effects of acetylcholine and barium.

In the case of Lergitin a concentration of 0.0125 μg per ml bath fluid inhibited the effect of a subsequent histamine dose (approxi-

mately 0.01—0.02 μg/ml bath fluid) completely whereas the concentration had to be raised about 400 times (5 μg per ml) to abolish the effect of equi-active amounts of acetylcholine, barium, choline, substance P, acetyl-β-methylcholine and nicotine. To abolish the effect of equi-active amounts of potassium chloride the concentration had to be raised about 2 000 times (25 μg Lergitin per ml) whereas the effect of pilocarpine was more easily counteracted — 2.5 μg Lergitin per ml suspension fluid was sufficient to annul the effect. The proper estimation of the Lergitin-dose necessary to abolish the effect of adenylic pyrophosphate was found rather difficult since the frequent addition of this substance rapidly destroyed the sensitivity of the intestine. It was, however, found that the doses of Lergitin which abolished the effect of histamine did not cause any visible decrease in the contraction height caused by adenylic pyrophosphate. In the case of substance P it was found that the effect of this substance was diminished to about 50 % when adding small, histamine-specific doses of Lergitin. After this initial reduction the effect of the substance P-preparation employed persisted even after the addition of rather large, "non-specific" amounts of antihistamine drug.

By raising the pH of the suspension fluid (Tyrode's solution) from the normal pH of 7.8 to about 9.0—9.5 through addition of small amounts of N NaOH a slow but strong contraction was obtained. This contraction could also be counteracted by means of Lergitin but only in high, "non-specific" concentrations.

From the above figures it was possible to calculate an approximate numerical expression of the relation between the specific, histamine antagonizing, and non-specific, antispasmodic, dose. If in the expression "specific: non-specific dose ratio" the concentration of Lergitin necessary to abolish the spasmogenic effect of histamine (*i. e.* the specific dose) was put equal to 1 and the non-specific dose to the concentration (in relation to the specific concentration 1) necessary to inhibit totally the action of each spasmogenic drug (acetylcholine, barium etc.) the following list was obtained:

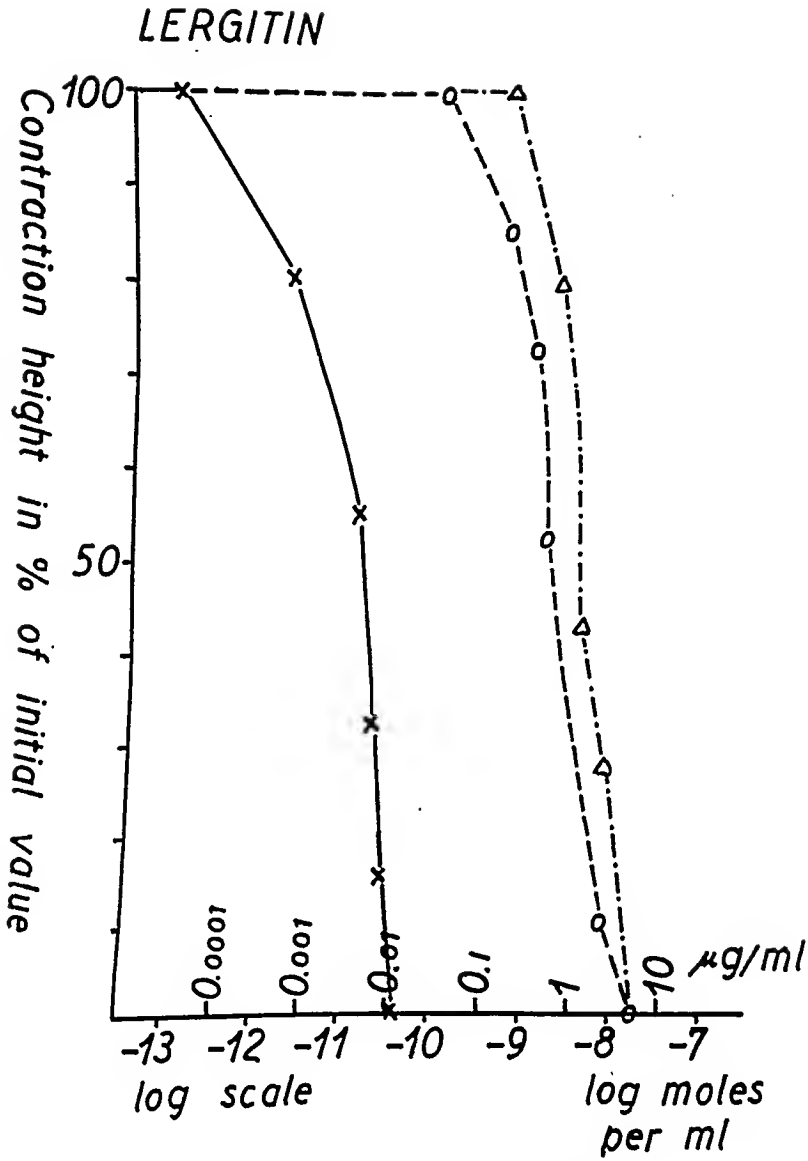

Fig. 10. Antihistamine and antispasmodic effect of Lergitin.

Abscisse: concentration of Lergitin (figures above the line: µg/ml intestinal bath fluid, figures below the line: log moles per ml).
Ordinate: contraction height induced by the spasmogenic substances after addition of Lergitin, expressed in % of initial contraction height (before the addition of Lergitin).

x———x———x histamine
o— — —o— — —o acetylcholine
△—·—·△—·—·△ barium chloride.

Spasmogenic substance	Specific: non-specific dose ratio
Acetylcholine chloride	1:400
Barium chloride	1:400
Choline chloride	1:400
Potassium chloride	1:2000
Pilocarpine hydrochloride	1:200
Substance P	1:>400
Acetyl-β-methylcholine chloride	1:400
Nicotine hydrochloride	1:400

From these records and from the diagrams (for example, see Fig. 10) it was easy to obtain a conception of the safety margin when using the antihistamine drug as "specific denominator" of histamine. Using the low concentrations here determined one might feel certain that the effect of other spasmogenic substances which might be present in extracts in addition to histamine will not be influenced. Even when raising the concentration of Lergitin *e. g.* 10 times over the lowest value obtained to extinguish the effect of histamine there still does not exist any danger that the effect of *e. g.* contaminating acetylcholine will be markedly influenced. It will be seen later that in extracts which obviously contain histamine as well as acetylcholine the smooth muscle stimulating effetct will be reduced to only a certain degree by adding the minimal (or a 5—10 times higher) dose of Lergitin whereas the remaining contraction, obviously due to acetylcholine will not be influenced unless the concentration of Lergitin is raised some 100 times or through the addition of atropine or through a previous splitting of the acetylcholine by cholinesterase from horse serum.

Using intestines with different sensitivity towards histamine (selected during the ordinary routine work in the laboratory) it was also possible to study if a certain amount of Lergitin was able to antagonize not only a certain dose of histamine but also to block the effect of higher, though equi-active, doses of histamine.

Usually the guinea-pig's isolated intestine gave strong contractions when the added amount of histamine gave a final concen-

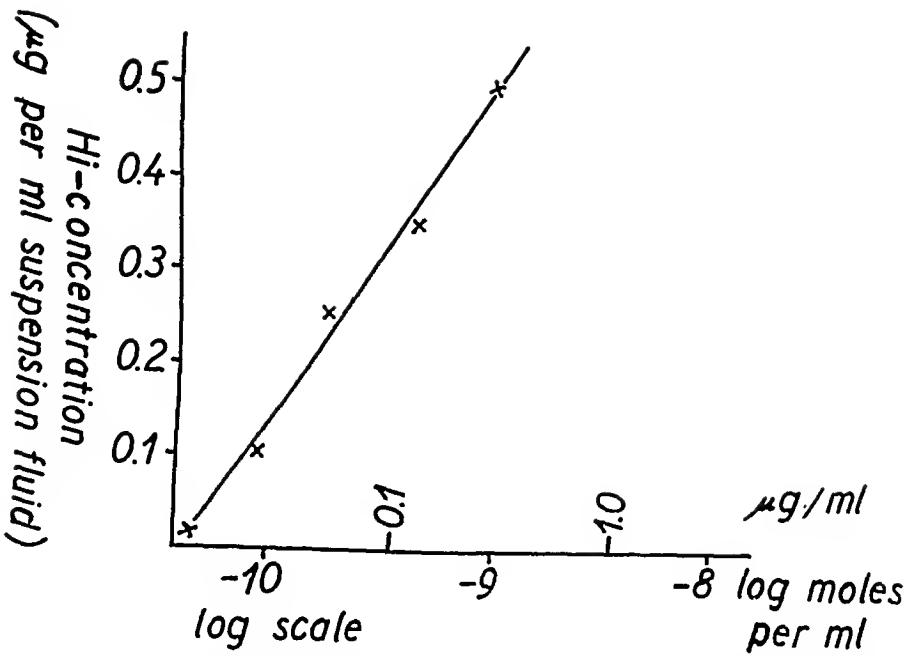

Fig. 11. The relation between different amounts of histamine (ordinate) necessary to evoke approximately the same contraction heights on intestines with different sensitivity and the amount of Lergitin (abscisse) necessary to abolish totally this effect.

tration in the bath fluid of 0.01 µg per ml (expressed as dihydrochloride salt). In some cases, however, a marked insensitivity towards histamine was observed, the intestines giving approximately the same contraction heights when adding 10—50 times larger histamine doses (giving final concentrations of 0.1—0.5 µg per ml). The diagram of Fig. 11 shows that there existed a close correlation between the amount of histamine necessary to elicit contractions of about the same height (equi-active doses) in different intestines and the amount of Lergitin which abolishes these histamine doses. Obviously a certain amount of antihistamine drug did not "cover" more than a certain amount of histamine.

The antispasmodic effect of atropine was tested in the same way as Lergitin. As could be expected, the inhibiting effect on acetylcholine was the most pronounced, a concentration of 0.0375 µg atropine sulfate per ml suspension fluid being sufficient to annul the effect of the subsequent acetylcholine-dose. The effect

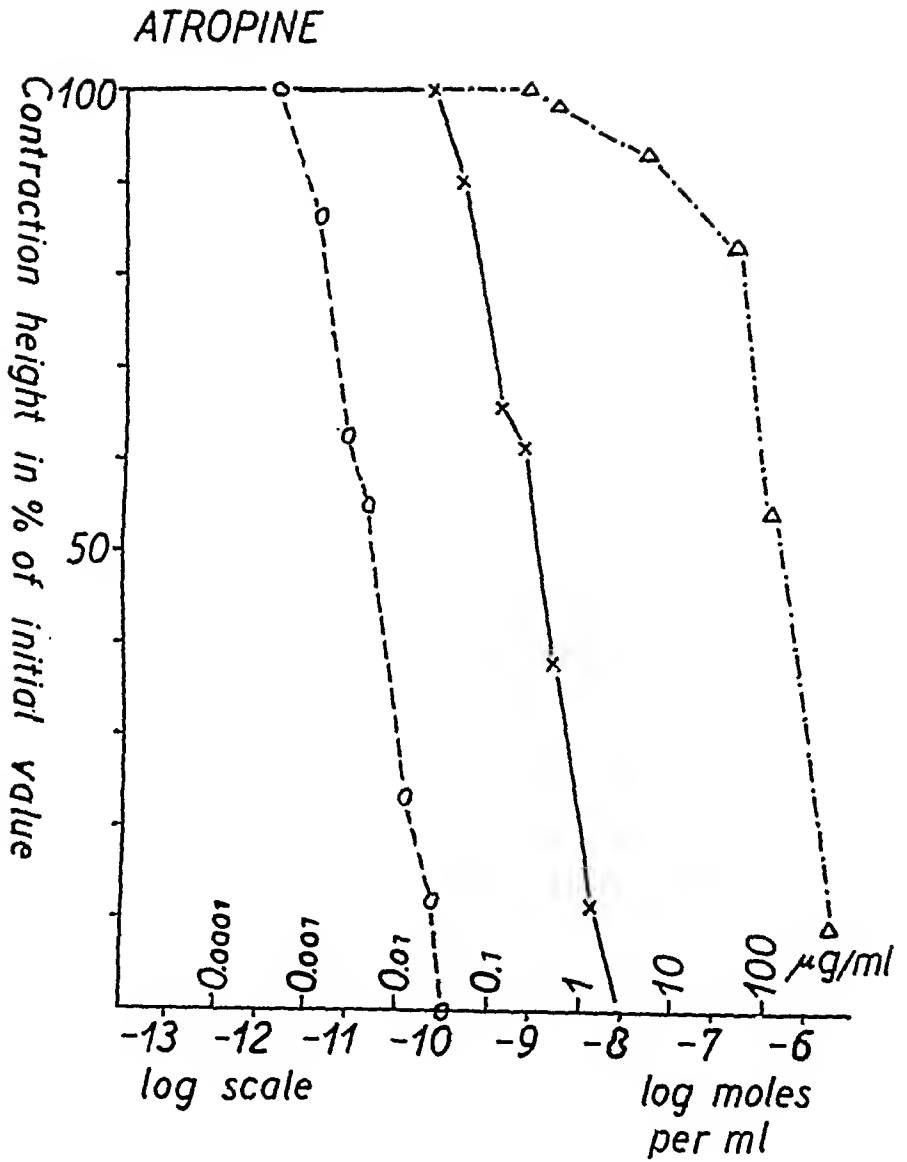

Fig. 12. Antispasmodic effect of atropine.

Abscisse: concentration of atropine (figures above the line: µg per ml intestinal bath fluid, figures below the line: log moles per ml).

Ordinate: contraction height induced by the spasmogenic substances after addition of atropine, expressed in % of initial contraction height (before the addition of atropine).

×———×———× histamine
o— — —o— — —o acetylcholine
△—·—·△—·—·△ barium chloride.

of equi-active doses of histamine was abolished at a concentration of 2.5 μg atropine per ml. $BaCl_2$ was not quite abolished even when the added amount of atropine was as high as 10 mg (giving a final concentration of 500 μg per ml suspension fluid). Obviously the anti-acetylcholine action of atropine in comparison with its general spasmodic action is fairly specific (cf. Fig. 12).

These experiments show the necessity of 1) using distinct concentrations of the antihistamine drug or atropine which do not simultaneously influence the effect of other spasmogenic substances and 2) standardizing the sensitivity of the intestines towards histamine (or acetylcholine). Only when these precautions are taken one may succeed in determining with this method whether the active substance is identical with histamine or acetylcholine. The specificity of the antihistamine drug here used (Lergitin) is obviously fairly high and the same statement holds true also for atropine as regards the effect on acetylcholine.

The blood pressure lowering action of histamine in cats in chloralose anaesthesia can also be blocked by an antihistamine drug (see LOEW's review, 1947). In order to obtain accurate values as to the specificity of Lergitin in this respect the dose necessary to abolish the histamine-effect was assayed. It was found that doses of Lergitin which completely neutralized the effect of histamine did not cause any significant change in the blood pressure fall caused by equi-active amounts of acetylcholine, choline, acetyl-β-methylcholine, or adenylic pyrophosphate. Usually 1—2 μg histamine dihydrochloride per kg body-weight caused a marked fall in blood pressure. In conformity with the findings in the intestinal bath experiments it was found also here that the effect of substance P was counteracted by Lergitin to a certain extent although it was not abolished (Fig. 13).

As regards the action of atropine on the blood pressure it is known that the depressor effect of histamine is not influenced by atropine (HUNT, 1917), an observation which has been confirmed in this investigation.

Through these two methods — testing on guinea-pig's isolated intestine and on cat's blood pressure with an antihistamine drug

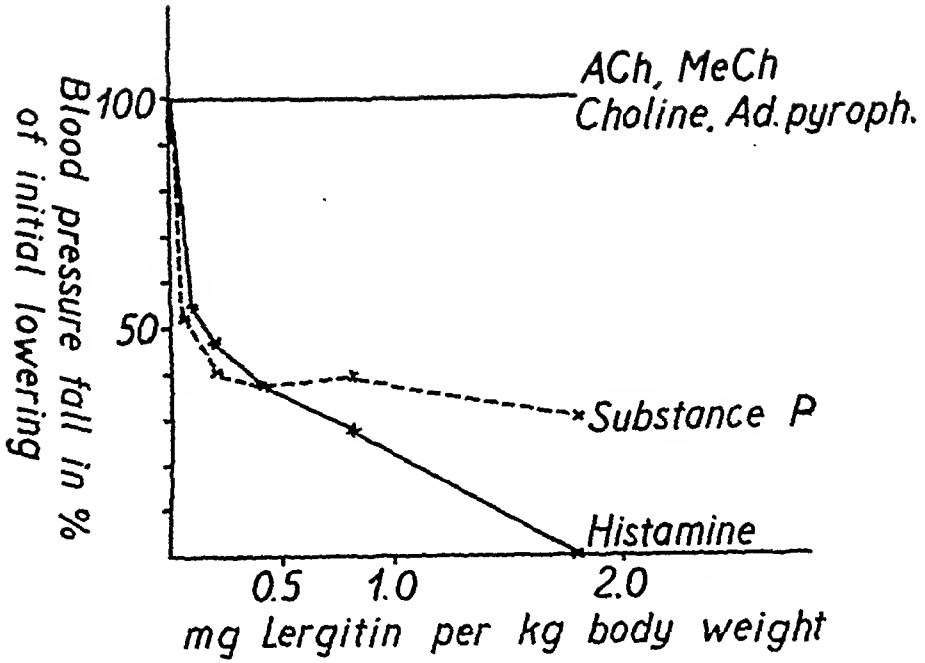

Fig. 13. A graphical representation of how the blood pressure lowering effect of histamine (1.5 μg/kg) was gradually abolished by increasing amounts of Lergitin. The depressor activity of equi-active doses of acetylcholine (ACh), acetyl-β-methylcholine (MeCh), choline and adenylic pyrophosphate (Ad.pyroph.) was not influenced wheras the effect of substance P was influenced to a certain extent.

as "specific denominator" — it seemed to be possible to verify the nature of unknown substanees of probable histamine identity. It seemed reasonable to argue that if an extract exerted a blood pressure lowering action and a stimulating effect on the isolated intestine and both these effeets could be neutralized by means of such doses of an antihistamine drug whieh in "test" experiments only counteracted the effeet of histamine, these aetions of the extraet must be attributed to the presence of histamine.

The assay of histamine and acetylcholine

When these faets had been established a further analysis of the depressor substance in extraets from eattle aorta was carried out.

As has been mentioned the pyridine-extracts from the fullers' earth used for adsorption of the depressor phase of the crude extracts were shown to contain histamine (SCHMITERLÖW, 1948). Not only was the effect on the isolated intestine and on blood pressure abolished by small doses of Lergitin but the extracts also showed a positive PAULY's reaction and a "triple response" when applied on the human skin according to T. LEWIS.

The extraction of the fullers' earth with pyridine at pH 8 will probably destroy some of the histamine (cf. "test" experiment, page 49). It was also found that when the fuller-treated aortic extracts were given to the intestinal bath there still remained smooth muscle stimulating substances in these extracts although they did not give any visible blood pressure fall. It could also be anticipated that if there was any acetylcholine present in the aortic extracts it would have been adsorbed to the fullers' earth but destroyed to a great extent at the subsequent elution at pH 8 since acetylcholine is fairly unstable in alkaline medium.

In order to avoid this error the aortic extracts were tested as such (crude extracts) on the isolated intestine. This way of proceeding involves, however, another error, since the presence of sympathomimetic substances will counteract the contraction caused by smooth-muscle stimulating agents. This error could, however, be rather exactly determined. From the figure given on page 76 it followed that these extracts contained approximately 2 μg sympathomimetic substance per g of fresh tissue (calculated as adrenaline). It was found that of these extracts an amount corresponding to *e.g.* 0.05 g gave a powerful contraction of the intestine (corresponding to the effect of 0.5 μg histamine). When adding this amount only about 0.1 μg sympathomimetic substance — presumably of nor-adrenaline-type — was added simultaneously. This means a concentration of 0.005 μg per ml of intestinal bath fluid. Using the same method as that described above for the estimation of histamine antagonizing action of Lergitin, the "anti-histamine" and "anti-acetylcholine" effect of nor-adrenaline could be determined. It was then found that nor-adrenaline was not capable of abolishing totally the effects of either histamine

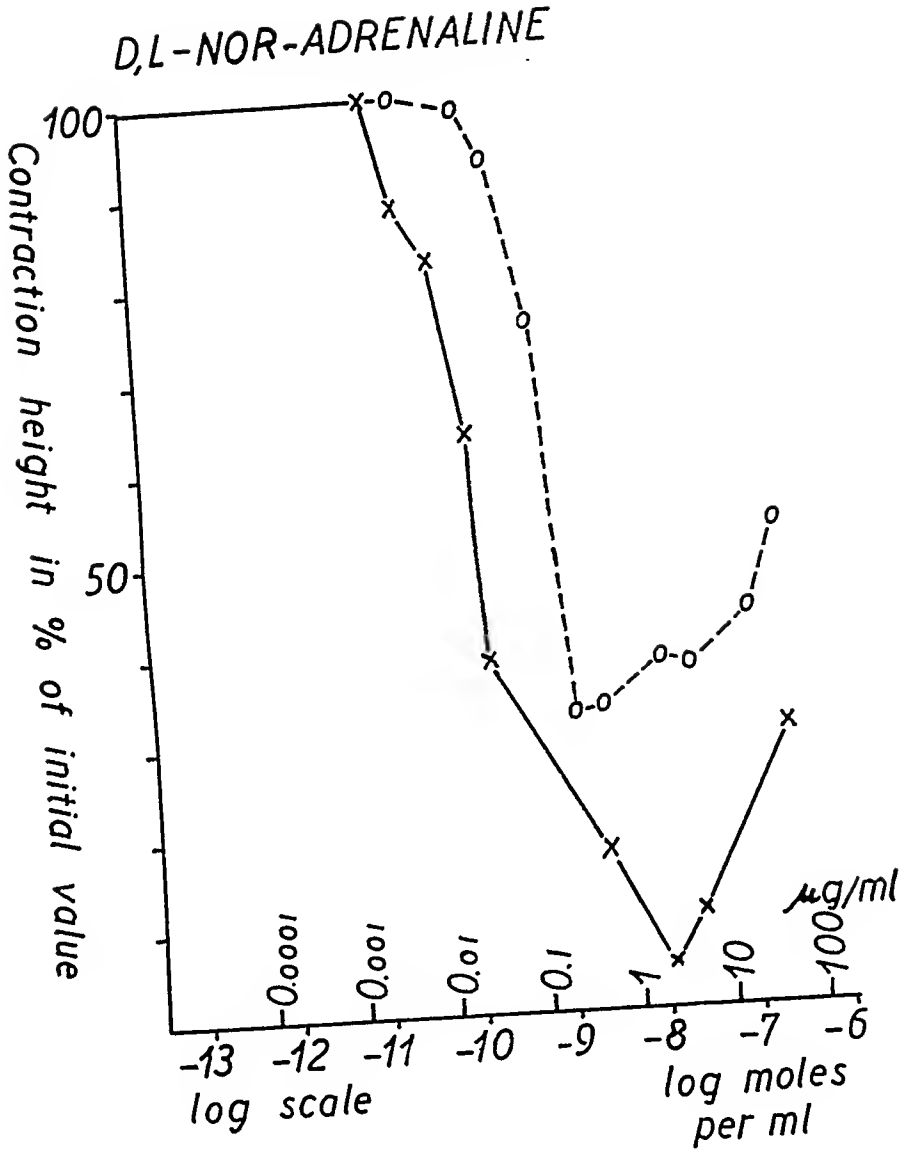

Fig. 14. The blocking effect of dl-nor-adrenaline on histamine and acetylcholine. Abscisse: concentration of dl-nor-adrenaline (figures above the line: µg per ml intestinal bath fluid, figures below the line: log moles per ml).

Ordinate: contraction height induced by the spasmogenic substances after addition of dl-nor-adrenaline, expressed in % of initial contraction height (before the addition of dl-nor-adrenaline).

×———×———× histamine
o— — —o— — —o acetylcholine

or acetylcholine, the following phenomenon being constantly observed: with increasing doses of nor-adrenaline there was at first a decrease in the response to histamine and acetylcholine. This decrease closely paralleled the inhibiting effect of adrenaline (determined in other experiments) but whereas adrenaline was capable of blocking the effects of these two agents a further increase of the added amounts of nor-adrenaline did not reduce the contractions to nil but on the contrary caused a slight rise (see Fig. 14). This effect was most pronounced as regards the action of acetylcholine. From Fig. 14 it can also be seen that the amount of nor-adrenaline present in the added extracts was too small to exert more than about 0—15 % reduction of the histamine effect, the effect of acetylcholine probably not being influenced at all.

The reliability of the assay was tested in the following two ways.

1. The amounts of smooth muscle stimulating agents in extract from human placenta was determined and then the same determinations were carried out with the same sort of extract with adrenaline and nor-adrenaline added (cf. test experiment described on page 44). It was then found that the assay of the amounts of smooth muscle stimulating substances gave values which were only 5—10 % lower than the values found in the placenta extract without the admixture of sympathomimetic substances. It may be pointed out here that the depressor and smooth muscle stimulating agent in the placenta extract consisted of acetylcholine and histamine in approximately the same concentrations, viz. 1 μg of each per g fresh tissue.

2. A mixture of the pure substances, histamine, acetylcholine and l-nor-adrenaline was made, corresponding to the concentration of these agents found in the extracts from cattle aorta. When assaying the amounts of histamine and acetylcholine in this mixture it was found that the presence of l-nor-adrenaline caused a lower value than was found when testing histamine and acetylcholine without the admixture of nor-adrenaline. This decrease, however, did not amount to more than 5—10 %.

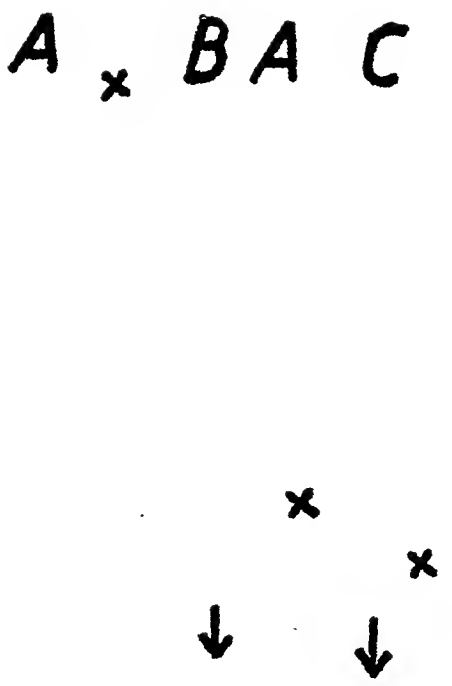

Fig. 15. Isolated guinea-pig's intestine. Bath volume 20 ml.

A. Extract from proximal portion of aorta from cattle, corresponding to 0.1 g fresh tissue.
B. 0.1 μg Lergitin.
C. 0.1 μg histamine (dose equi-active with 0.1 g aorta).
× = movement of intestine due to emptying of the bath.

When this had been tested the amounts of smooth-muscle stimulating substances in extracts from aortic wall of cattle was determined.

When adding "specific" doses of Lergitin to the intestinal bath only a part of the contraction caused by the extract was blocked, a small contraction still remaining, although the action of equi-active amounts of histamine was quite abolished (see Fig. 15). By adding small, "acetylcholine-specific" doses of atropine this remaining contraction could be quite abolished. Fig. 16 demonstrates an experiment in which an "acetylcholine-specific" dose

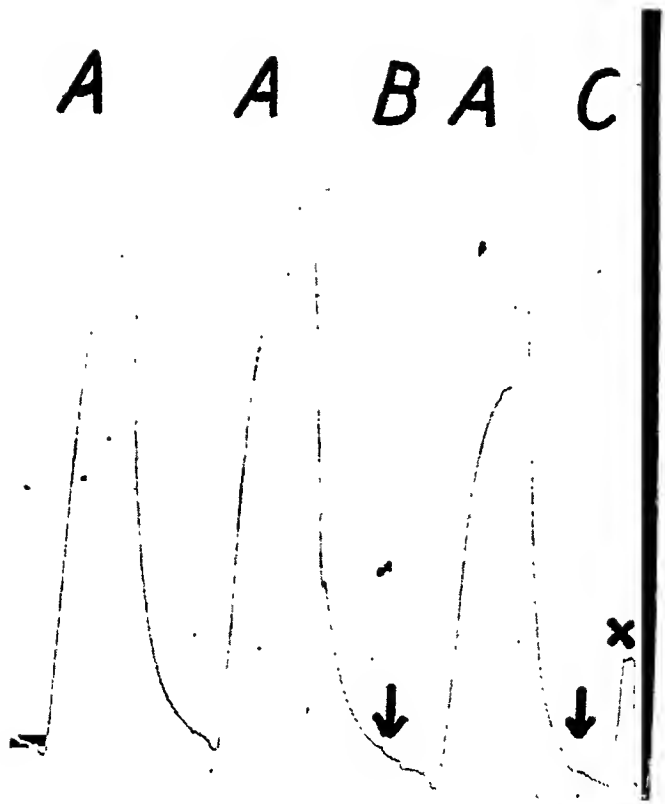

Fig. 16. Isolated guinea-pig's intestine. Bath volume 20 ml.

A. Extract from proximal portion of aorta from cattle, corresponding to 0.1 g fresh tissue.
B. 1 μg atropine.
C. (15 μg acetylcholine.
× = movement of intestine due to emptying of the bath.

of atropine was added to the bath and then the aortic extract. In this case a slight decrease in activity was observed, whereas a subsequent dose of acetylcholine was totally abolished.

The determination of the amounts of histamine and acetylcholine have been carried out in three different parts of aorta from cattle, viz. the proximal portion (first 10 cm of aorta), the posterior thoracic portion (*i. e.* the remainder of the thoracic aorta) and the abdominal part. The figures obtained are given below.

Aorta	Histamine µg/g fresh tissue	Acetylcholine µg/g fresh tissue
Proximal portion	9	0.4
Posterior thoracic portion	14	0.8
Abdominal portion	11	0.7

There was a distinet differenee in eontent between the three different parts of aorta. This will be diseussed in eonneetion with the values obtained for the content of pressor substanee in these three parts (ef. page 83).

These figures differ from the figure given in a previous paper (SCHMITERLÖW, 1948). This is due to several facts: 1. The fullers' earth employed in these investigations did not remove more than a part of the depressor and smooth-musele stimulating substances from the crude aortie extracts, the fuller-treated extracts still giving a strong contraetion of the isolated intestine, although their depressor aetion on blood pressure was obviously hidden by the presenee of the pressor substance. 2. The pyridine-elution may be ineomplete although it gave higher yields than when aleohol or Ringer's solution or a hypertonie salt solution were used as elution fluids. 3. The elution at a sligthly alkaline pH destroys some of the present histamine and probably most of the prevailing acetylcholine.

It may be pointed out that the determinations of the smooth-muscle stimulating agents must be earried out as soon as possible after the preparation of the extraets. If the extracts are allowed to stand the eontent of aeetylcholine is fairly rapidly diminished whereas the histamine eontent seemed to keep at a constant level even after about 2—3 weeks.

It may further be pointed out that the small eontraction remaining after the addition of "speeifie" doses of Lergitin eould be abolished by adding small amounts of fresh horse serum to the extracts, obviously due to an enzymatie break-down of the acetyleholine.

It was thus shown that the extracts from cattle aorta contain both histamine and acetylcholine.

When the nature of the depressor phase was thus determined a possible explanation might be offered for the apparently contradictory experimental results regarding the nature of the sympathomimetic substance in aortic extracts.

The secondary rise following the blood pressure fall after injecting histamine has been shown to be due to an increased output of adrenaline from the adrenals (DALE, 1920; KELLAWAY and COWELL, 1922; HOGBEN, SCHLAPP and MACDONALD, 1924; BURN and DALE, 1926; FELDBERG, 1929). When injecting an extract which is not completely free from histamine (giving a small initial fall of blood pressure) this histamine may be enough to cause an output of adrenaline from the adrenals of the test-animal itself and then to the pressor action of the extracts is added the action of this adrenaline which may obviously be quite enough to cause a relaxation of the non-pregnant uterus. And furthermore even if there is no visible depressor effect but only a distinct rise of blood pressure there may still be enough histamine to cause an output of adrenaline. This histamine is certainly masked by the pure rise in blood pressure but its existence may be proved on the isolated intestine of the guinea-pig. The following experiment was undertaken in order to prove this supposition. The effects of histamine, adrenaline and nor-adrenaline on blood pressure and non-pregnant uterus in situ were tested first separately and then a mixture of histamine and nor-adrenaline was injected. Fig. 17 shows the result. The injected adrenaline as well as the natural adrenaline produced by the test-animal itself as a result of the histamine-action, both produced a relaxation of the uterus, whereas pure nor-adrenaline did not exert any visible action on the uterus. The mixture of histamine and nor-adrenaline caused a pure blood pressure rise (except a small notch just at the beginning of the elevation) but nevertheless there was a distinct relaxation of the uterus. It will be noticed in Fig. 17 that the relaxation in the case of injecting histamine or histamine + nor-

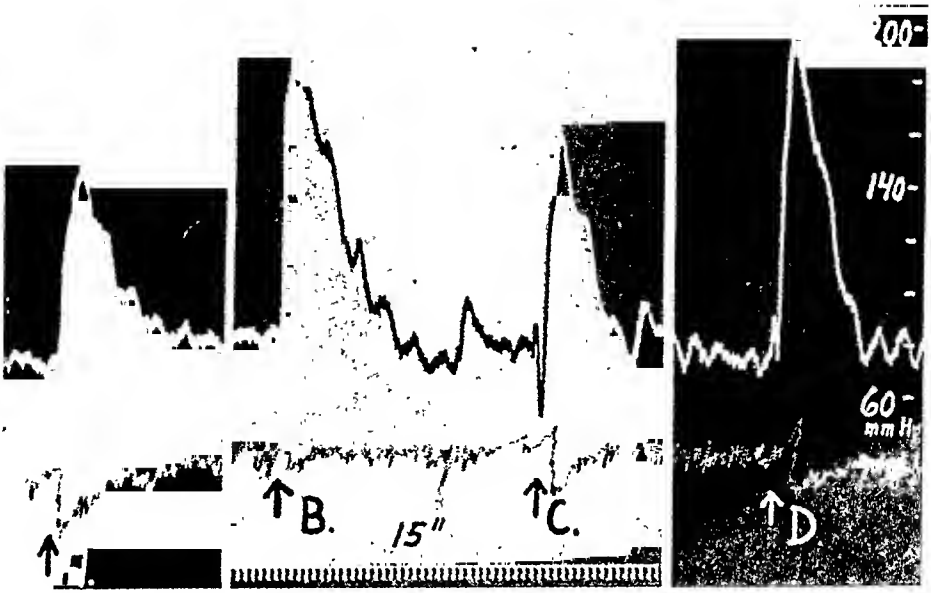

Fig. 17. Cat, blood pressure (upper curve), non-pregnant uterus in situ (lower curve). Adrenals intact. Chloralose i. m.

A. 1 µg adrenaline.
B. 1 µg dl-nor-adrenaline.
C. 1 µg histamine.
D. 1 µg histamine + 1 µg dl-nor-adrenaline, given simultaneously.
Time 15 secs.

adrenaline occurred after a certain delay as compared with the injection of pure adrenaline where the relaxation came on almost instantaneously.

This experiment and the confusing results with the aortic extracts seemed to necessitate the use of completely adrenalectomized test-animals in order to avoid the admixture of adrenaline from the test-animals. The following routine method was adopted. The animals were adrenalectomized prior to the beginning of the experiments and were then given Lergitin (2—6 mg/kg i. v. and 2 mg/kg i. m.) and atropine (0.25 mg/kg i. v.). After this treatment even the crude extracts of cattle aorta often gave a pure pressor response and if this was not the case they were treated with fullers' earth as described above. Neither Lergitin nor atro-

pine will decrease the sensitivity towards the sympathomimetic substances and if the treatment of the extracts with fullers' earth thus can be avoided this is an advantage, since this purification method always caused a slight loss of pressor substance.

C. The pressor (sympathomimetic) activity

In adrenalectomized cats which had received Lergitin and atropine the crude extracts often gave a pure pressor response. If there still was a small initial lowering of the blood pressure this effect could be removed by treating the extracts with fullers' earth as described above. Although the treatment with fullers' earth regularly caused a loss in activity of pure watery solutions of adrenaline and nor-adrenaline the treatment of the extracts not always caused a loss in activity but on the contrary an increase (Fig. 18 A and B). This was probably due to the fact that when the contaminating depressor substances were partly removed by adsorption to the fullers' earth the relation between depressor and pressor substances in the extracts was changed so as to allow the pressor substance to come into more visible action.

The amount of pressor substance in extracts from the thoracic part of aorta equalled approximately 2 μg l-adrenaline or 1.25 μg l-nor-adrenaline per g fresh tissue.

The pressor effect of the extracts was enhanced by cocaine.

Treating the extracts with iodine at pH 8 rapidly destroyed the pressor activity of the extracts (Fig. 18 C).

The pressor activity was destroyed to a great extent when the extracts were boiled in normal alkaline solution for 10 minutes. The same treatment in normal acid solution also caused a certain loss of activity which was, however, not very great. It may be pointed out in this connection that if the extracts were treated with alkali they soon acquired a slight red colour, just as alkali-treated solutions of adrenaline or nor-adrenaline.

If the extracts were dialyzed in a cellophane bag for 36 hours against distilled water which was changed twice during this pro-

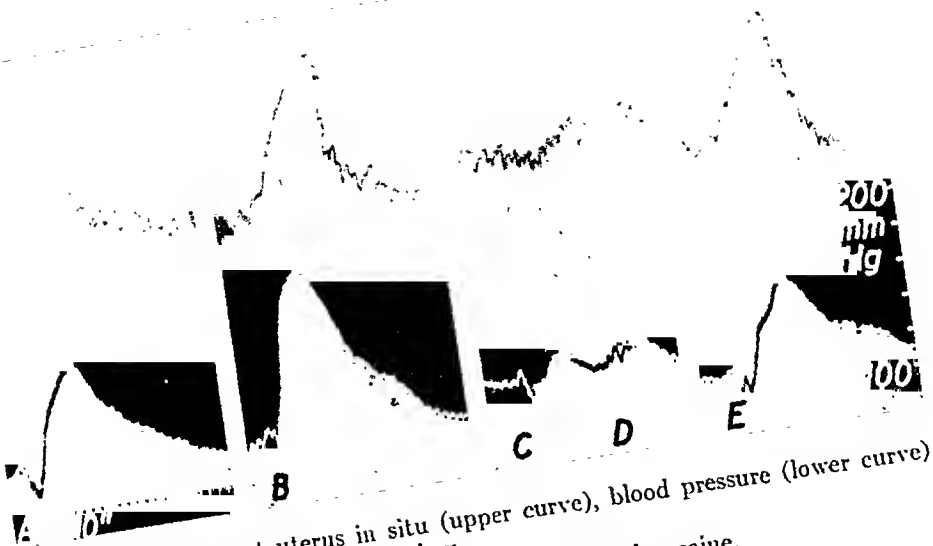

Fig. 18. Cat. Pregnant uterus in situ (upper curve), blood pressure (lower curve). Both adrenals removed. Chloralose i. m.
After previous administration of Lergitin, atropine and cocaine.
A. Extract from cattle aorta, corresponding to 2 g fresh tissue.
B. The same, treated twice with fullers' earth (5 mg KN 11 C per ml extract).
C. The same as A. treated with iodine at pH 8.
D. The same as A. dialyzed for 36 hours.
E. The dialysate from A.
Time 10 secs.

cedure almost all of the pressor activity could be recovered from the dialysate (Fig. 18 A, D and E).

After 0.5 mg of dihydroergotamine methansulfonate per kg body-weight intravenously the blood pressure raising effect was diminished but not reversed. After 15 mg/kg of dibenamine the effect was highly reduced but not reversed (Fig. 19).

The blood pressure raising effect was rapidly destroyed when the extracts were treated at pH 7.0 with small amounts of tyrosinase prepared from meal-worms or catechol oxidase from potatoes according to the methods described by PUGH (1930) — meal-worms — and SZENT-GYÖRGYI (1929) — potatoes. The effect of adding these enzyme preparations to solutions of adrenaline and nor-adrenaline was studied by HEIRMAN (1937) and BACQ (1937, 1938). They found that the normal action of adrenaline and nor-adrenaline disappeared in a few minutes. After some time —

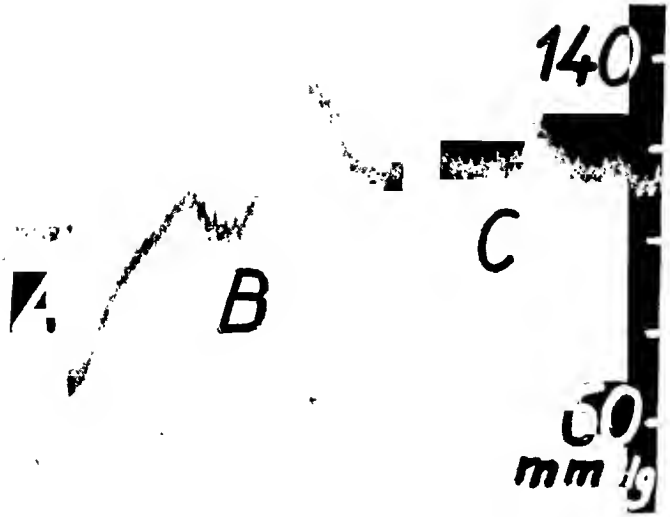

Fig. 19. Cat, blood pressure. Chloralose i. m. Both adrenals removed.
After previous administration of Lergitin, atropine and 15 mg/kg dibenamine.
A. 3 μg adrenaline.
B. 3 μg l-nor-adrenaline.
C. Extract from cattle aorta, corresponding to 3 g fresh tissue.
Time 10 secs.

45—120 minutes (or more) — the adrenaline-solution suddenly exerts a powerful vaso-dilator action which then gradually disappears whereas the oxidation of nor-adrenaline does not result in the production of an inhibitory substance. It was thought that this reaction may serve as a means of securing the nor-adrenaline-identity. It was, however, shown by BLASCHKO and SCHLOSSMANN (1938) that during the oxidation of adrenaline by pure preparations of catechol oxidase neither pressor nor depressor activity could be observed. The preparation of tyrosinase from potatoes according to the method described by EVANS and RAPER (1937) is stated to contain peroxidase in addition and it could be thought that this enzyme would be responsible for the actions deseribed by HEIRMAN and BACQ. BLASCHKO and SCHLOSSMANN (1940), however, found that in the inactivation of adrenaline by peroxidase the adrenaline loses excitatory and inhibitory properties simultaneously.

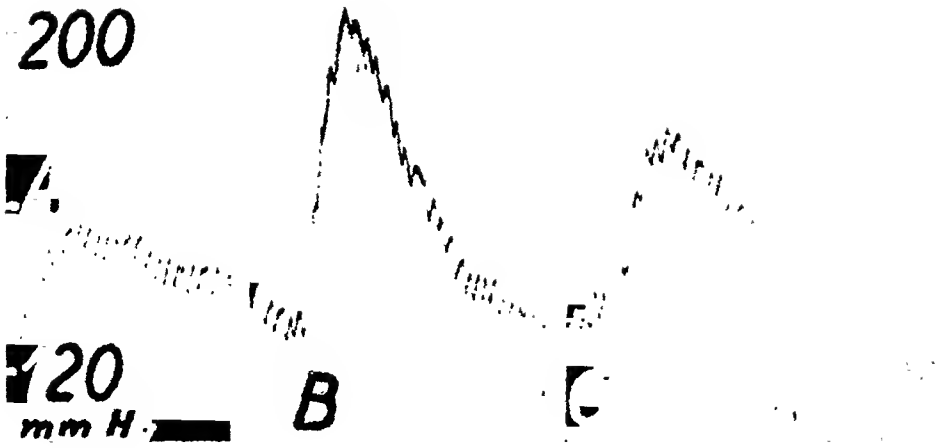

Fig. 20. Cat, blood pressure. Both adrenals removed. Chloralose i. m.
After previous administration of cocaine.

A. Extract from proximal portion of cattle aorta, treated twice with fullers' earth (5 mg KN 11 C per ml extract), corresponding to 1 g fresh tissue.

B. Extract from posterior thoracic portion of cattle aorta (purification and amount as in A).

C. Extract from abdominal portion of cattle aorta (purification and amount as in A).

Time 10 secs.

The effect of these enzyme preparations was tested on pure solutions of adrenaline and l-nor-adrenaline and on purified extracts of cattle aorta. The pressor acitivity of these three solutions was rapidly destroyed. No reversal of the adrenaline action could, however, be obtained even if the enzyme preparation was allowed to act for more than 5 hours. Simultaneously with the loss of pressor activity of adrenaline the inhibitory action on the non-pregnant uterus disappeared.

When testing on blood pressure the content of pressor activity in extracts from different parts of the cattle aorta it was found that the proximal portion of the thoracic aorta (for explanation see page 72) contained rather small amounts of pressor activity. the abdominal portion somewhat more and the lower thoracic part most (Fig. 20).

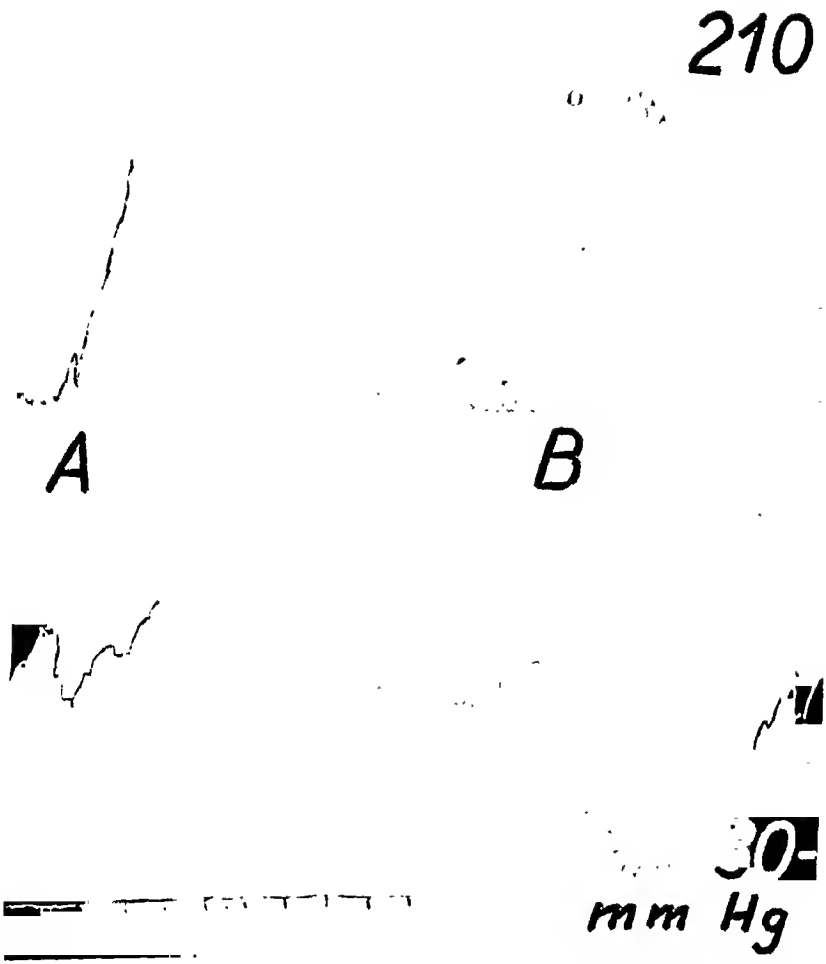

Fig. 21. Cat, blood pressure (upper curve), non-pregnant uterus in situ (lower curve). Both adrenals removed. Chloralose i. m.

After previous administration of Lergitin, atropine and cocaine.

A. Extracts from cattle aorta, corresponding to 1 g fresh tissue.

B. 2 μg adrenaline.

Time 10 secs.

Non-pregnant and pregnant cat's uterus in situ

In adrenalectomized cats the aortic extracts gave no visible change of the tone and rhythm of the non-pregnant uterus whereas equi-pressor amounts of l-adrenaline invariably caused a more or

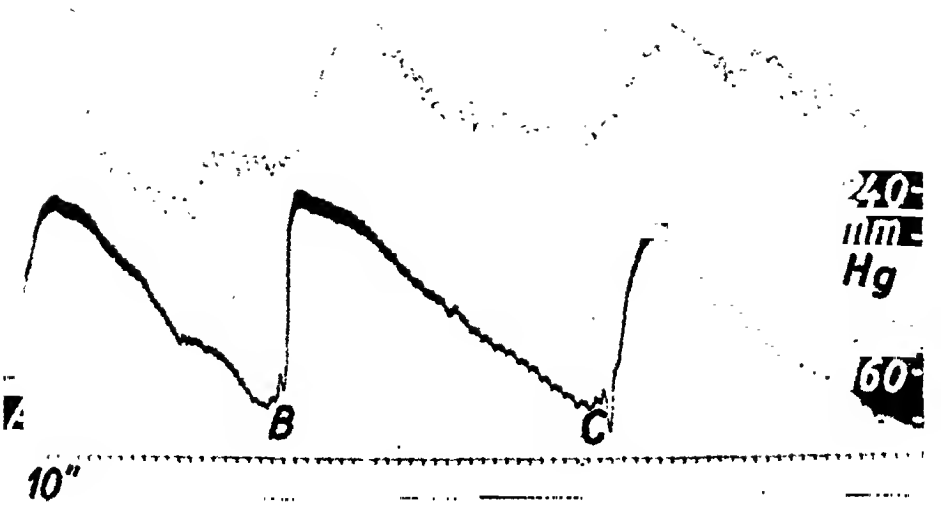

Fig. 22. Cat, pregnant uterus in situ (upper curve), blood pressure (lower curve). Both adrenals removed. Chloralose i. m.
After previous administration of Lergitin, atropine and cocaine.

A. 2 μg adrenaline.
B. 2 μg l-nor-adrenaline.
C. Aortic extract from cattle aorta, corresponding to 2 g fresh tissue, treated twice with fullers' earth (5 mg KN 11 C per ml extract).
Time 10 secs.

less marked relaxation (Fig. 21). dl-Nor-adrenaline was never found to give any significant relaxation whereas large doses of l-nor-adrenaline sometimes caused a slight inhibition of the movements. It might be pointed out that if an extract gave a small initial lowering of the blood pressure a slight excitatory effect was observed on the uterus. This was probably due to the presence of histamine in the extracts since histamine causes a tonic contraction of the uterus (cf. FELDBERG and SCHILF, "Histamin", 1930).

On the pregnant cat's uterus the extracts gave a marked increase of the tone. As has been pointed out before (page 53) there is sometimes a certain and fairly evident difference between the action of adrenaline and nor-adrenaline on the pregnant uterus,

the relaxation phase after the sharp initial rise being more prolonged in the case of nor-adrenaline. In these cases where this difference was observed it was also found that the purified extracts (or histamine- and acetylcholine-antagonized extracts) caused an excitatory response which differed from the effect of adrenaline but resembled the action of nor-adrenaline (Fig. 22).

Heart rate

The extracts giving pure pressor responses gave a distinct increase in heart rate, similar to the effect of adrenaline and nor-adrenaline.

Fluorescence test

The purified extracts showed a bluish fluorescence of their own which could be considerably reduced after the treatment of the extracts with $Al(OH)_3$ at pH 4. After this treatment there was only a faint bluish tint in the ultra-violet light.

When comparing the fluorescence obtained one minute after addition of alkali to solutions of l-adrenaline, l-nor-adrenaline and aortic extracts in equi-pressor concentrations the adrenaline solution showed the bright green fluorescence described by GADDUM and SCHILD (1934). l-Nor-adrenaline gave a very slight fluorescence and the aortic extracts only showed a very slight increase in the bluish colour but no change to green fluorescence.

Colour reaction

The purified extracts were diluted so as to correspond approximately to a concentration, corresponding to 0.5 μg adrenaline per ml and were then tested according to the colour method, originally described by SHAW (1938). For comparison pure watery solutions of l-adrenaline and l-nor-adrenaline were used. The samples of l-adrenaline, l-nor-adrenaline and aortic extract were divided in two parts and the difference in colour between alkali-treated and not alkali-treated samples was studied colorimetrically. The extracts from cattle aorta as well as the pure solution of l-nor-adrenaline did not show any difference in colour, the

colorimeter readings being, as a matter of fact, almost exactly the same for both samples whereas in the case of l-adrenaline there was a pronounced difference, the alkali-treated portion showing a rather stronger colour.

Difference in activity in extracts from different parts of the cattle aorta

It has been pointed out above that both the amounts of depressor and pressor activity is different in different parts of the aorta. There was a close resemblance between the depressor and pressor effect in this respect and for both of them the following succession was obtained:

a) proximal portion
b) abdominal portion
c) posterior thoracic portion

the posterior thoracic portion containing most of both depressor and pressor activity.

The suggestion of EULER that the active substance in extracts from various organs is connected with nervous structures and the presence of the sympathomimetic, nor-adrenaline-like substance in adrenergic nerves gave the basis for the following investigation.

The difference in activity in extracts from the various parts of aorta might be due to a different innervation of these parts. A histological examination (Professor A. PALMGREN) revealed the following fact. The proximal portion of the cattle aorta contained very few nerve stems, the abdominal portion somewhat more whereas the posterior thoracic portion contained quite a number of nerve stems. These nerve stems run in the adventitia layer and give off branches to the media.

It seemed likely that this difference in innervation was responsible for the different amounts, not only of the pressor substance, but also of the depressor substance (histamine and acetylcholine).

In order to elucidate further this connection between the amount of nerves present in the extracted tissue and the amount of vaso-active substances the following experiment was performed.

The intima layer was gently separated from the rest of the aortic wall. This procedure was, however, not so simple in the case of cattle aorta, since the intima layer was rather firmly attached to the media (in the case of horse aorta this separation procedure was much easier). The aorta was cut open and was then spread flat on the table. In one of the ends a small incision was made with a razor just under and parallel to the intima. Using this freed part of the intima as a handle this layer could then be gently pulled off as a thin film but there were always small pieces of the media attached to this "film". The remainder of the aortic wall was then divided in two layers by means of the razor. Three layers were thus obtained, viz. the "intima layer" (with some attaching strips of the media), a middle layer consisting mainly of the tunica media and an outer layer consisting of adventitia and a part of the tunica media (histological examination).

These three layers were then extracted separately. When testing the effect of these layers it was found that the intima layer contained very little pressor activity, the middle layer contained some and the outer layer most pressor activity.

In this connection the amounts of histamine and acetylcholine in these three layers were also tested (isolated guinea-pig's intestine). It was found that the same order of activity held true also in regard to these substances.

	Histamine µg/g	Acetylcholine µg/g
"Intima layer"	2.5	0.05
Middle layer	6.5	0.2
Outer layer	10.0	0.5

(extracts from posterior thoracic portion)

According to the literature the intima contains few, if any nerve fibres, nor could any fibres be observed when examining the histological preparations from the aorta. In the media there was an abundance of fine nerve fibres and in the adventitia layer there were larger nerve stems. This distribution of the nerves in the aorta seems to coincide with the distribution of vaso-active substances.

Extracts were also made from calf aorta and from calf foetus aorta. The amount of pressor substance seemed to be approximately the same as in the adult aorta, whereas the amount of histamine and acetylcholine was considerably less (calf foetus aorta: 1.5 μg histamine, calf aorta: 3 μg histamine per g fresh tissue. Only traces of acetylcholine were found).

II. Aorta from other animals

In the beginning of this investigation cattle aorta was used exclusively. This choice of material offered a possibility to study both depressor and pressor activity in blood vessel extracts. The investigations of pressor (sympathomimetic) activity in aortic extracts from other species (horse, pig, dog) was found, however, to be much easier, since they were found to contain considerably smaller amounts of depressor substances. This was especially true in the case of horse aorta.

The crude extracts from horse aorta gave, as a rule, a pure rise in blood pressure. Only occasionally was there a very small initial lowering of the blood pressure, indicating, however, the presence of depressor substances. The crude aortic extracts from pig and dog usually gave a small initial lowering, which could easily be counteracted through the administration of Lergitin and atropine.

A. The depressor activity

When testing the extracts on the isolated guinea-pig's intestine it was found that the addition of the same amount of these extracts as of extracts from cattle aorta (corresponding to 0.03—0.1 g fresh tissue) did not cause any visible contraction of the intestine at all. The added amount had to be raised so as to correspond to about 2 g of fresh tissue in order to give any contraction. When this amount was added a fairly high concentration of sympathomimetic substance was also added (approximately

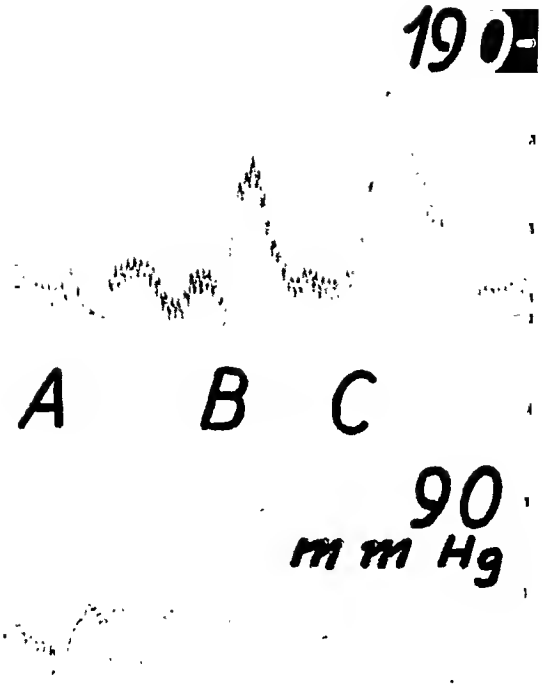

Fig. 23. Cat, blood pressure (upper curve), non-pregnant uterus in situ (lower curve). Both adrenals removed.
After previous administration of cocaine.
A. Extract from intima layer of horse aorta, corresponding to 1 g fresh tissue.
B. Extract from middle layer of horse aorta corresponding to 1 g fresh tissue.
C. Extract from outer layer of horse aorta, corresponding to 1 g fresh tissue.

giving a concentration of 0.3 μg per ml suspension fluid). Judging from Fig. 14 this would reduce the activity of histamine to approximately 20 %. Even if this was taken into consideration the amount of histamine — specifically blocked by Lergitin — would not exceed 0.3 μg per g tissue. The amount of acetylcholine present was very low, probably not exceeding 0.05 μg per g fresh tissue.

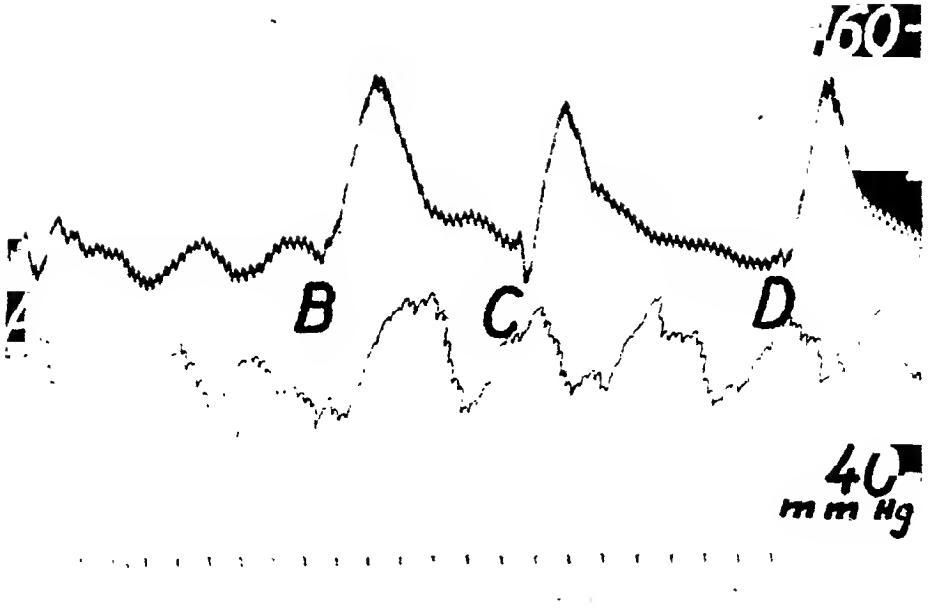

Fig. 24. Cat, blood pressure (upper curve), non-pregnant uterus in situ (lower curve). Both adrenals removed. Chloralose i. m.
After previous administration of 0.5 mg dihydroergotamine per kg body-weight.
A. 1 μg adrenaline.
B. 1 μg l-nor-adrenaline.
C. Extract from dog aorta, corresponding to 1 g fresh tissue.
D. Extract from horse aorta, corresponding to 1 g fresh tissue.
Time 10 secs.

The determination of histamine and acetylcholine content of aortic extracts from pig and dog revealed approximately 0.9 μg histamine per g fresh tissue and only small traces of acetylcholine.

B. The pressor (sympathomimetic) activity

Since the extracts from horse aorta almost always gave a pure pressor effect it was not neccessary to perform any purification procedure with fullers' earth. The determination of the amount and nature of the pressor substance was thus rendered more easy. It was found that the pressor activity corresponded to 3 μg adrenaline or 1.75 μg l-nor-adrenaline per g fresh tissue.

Also in the case of horse aorta a separation of three different layers of the aortic wall was performed and extracted separately.

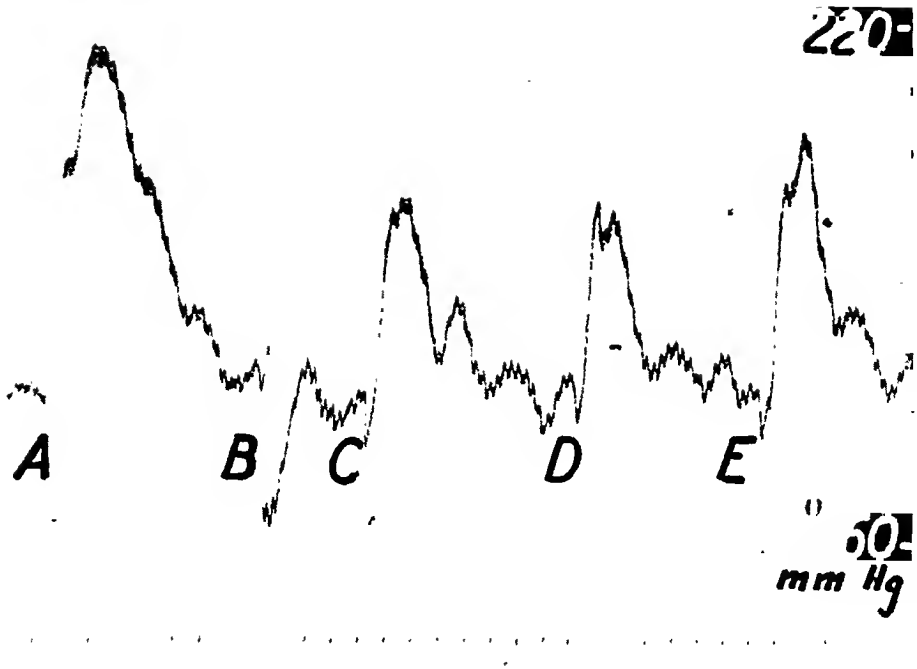

Fig. 25. Cat, blood pressure. Both adrenals removed. Chloralose i. m. After previous administration of cocaine.

A. 3 μg l-nor-adrenaline.
B. 3 μg adrenaline.
C. Extract from dog aorta, corresponding to 3 g fresh tissue.
D. Extract from pig aorta, corresponding to 3 g fresh tissue.
E. Extract from horse aorta, corresponding to 3 g fresh tissue.
Time 10 secs.

The separation of the intima layer from the rest of the aortic wall was here very simple and it was possible to pull off the intima layer as a thin film without getting any visible admixture of media tissue. The same order of activity was obtained as in the case of cattle aorta (Fig. 23).

The pressor activity in aortic extracts from pig and dog equalled 2.5 μg adrenaline per g.

The aortic extracts from these animals increased the heart rate and did not relax the non-pregnant uterus of the cat. Their pressor acitivity was not reversed by ergotamine (Fig. 24) or diben-

amine. They did not give the green fluorescence of adrenaline and in SHAW's colour reaction their colour was not increased by addition of alkali.

One of the experiments might be especially mentioned. In one of the experimental cats it was found that adrenaline constantly gave a pure blood pressure fall whereas the same doses of dl-nor-adrenaline or l-nor-adrenaline invariably caused a rise in blood pressure. Extract from horse, pig, and dog aorta also invariably caused a rise in blood pressure (Fig. 25). In this cat the adrenaline reversal was, so to speak, natural for the doses employed.

Chapter VII

Pressor and depressor activity of extracts from arteries and veins

When the nature and amounts of depressor and pressor substances in aortic wall had been investigated extracts were also made from other blood vessels. In the beginning of this investigation blood vessels from cattle were used. In conformity with the findings in regard to the cattle aorta the extracts from smaller vessels of cattle were also found to contain large amounts of depressor substances whereas blood vessels from the horse contained only very small amounts of depressor substances. The investigation of the pressor activity was thus rendered more easy when using blood vessels from the horse.

The extracts from various blood vessels from the horse often exerted a pure rise in blood pressure and if there was a small initial fall this could be completely blocked by previous administration of Lergitin and atropine.

Coronary arteries. The presence of a sympathomimetic substance in extracts from the heart has been investigated by several authors. Some discussion has arisen concerning the nature of this substance but from EULER's investigations (1946 c, d) it is now evident that the sympathomimetic substance in extracts from *frog's* heart is identical with adrenaline whereas the *mammalian* heart contains a substance with the properties of nor-adrenaline. BACQ and FISCHER (1947) found, however, only an adrenaline-like substance in extracts from human coronary arteries.

The extracts from coronary arteries from the horse exerted an action which mimicked the action of nor-adrenaline. The rise in blood pressure was not accompanied by any change in tone and

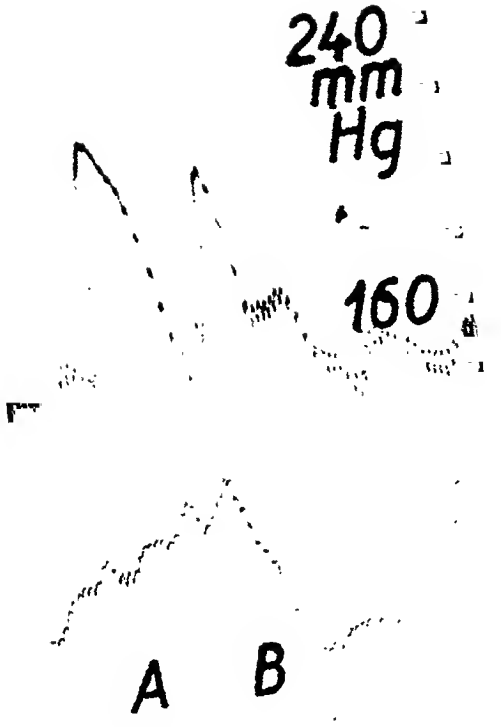

Fig. 26. Cat, blood pressure (upper curve), non-pregnant uterus in situ (lower curve). Both adrenals removed. Chloralose i. m.
After previous administration of Lergitin and atropine.
A. Extract from horse coronary vessels, corresponding to 1 g fresh tissue.
B. 2 µg adrenaline.
Time 10 secs.

rhythm of the non-pregnant uterus (Fig. 26), the pressor aetivity was not reversed by dihydroergotamine (Fig. 27 C), the SHAW reaetion did not reveal any increase in eolour when adding alkali and the extraets did not give the green fluorescence in ultra-violet light when adding alkali.

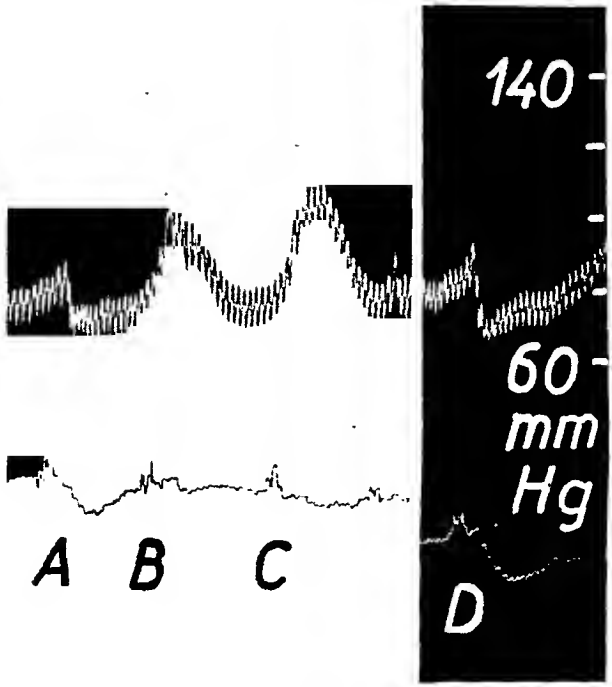

Fig. 27. Cat, blood pressure (upper curve), non-pregnant uterus in situ (lower curve). Both adrenals removed. Chloralose i. m.
After previous administration of Lergitin, atropine and 0.5 mg/kg dihydroergotamine.
A. Extract from horse renal arteries, corresponding to 1 g fresh tissue.
B. Extract from horse mesenteric arteries, corresponding to 1 g fresh tissue.
C. Extract from horse coronary arteries, corresponding to 1 g fresh tissue.
D. 4 μg adrenaline.
Time 10 secs.

The amount of pressor substance in coronary arteries corresponded to approximately 3 μg adrenaline per g.

Mesenteric arteries. Extracts from mesenteric arteries from the horse exerted an action which biologically, physically and chemically closely resembled that of nor-adrenaline (Fig. 28 A and Fig. 27 B). The amount corresponded to 2.5 μg adrenaline per g fresh tissue.

Femoral and common carotid arteries. Extracts from these vessels also contained a pressor substance which behaved like nor-adrenaline, the amount corresponding to approximately 0.75 μg adrenaline per g fresh tissue.

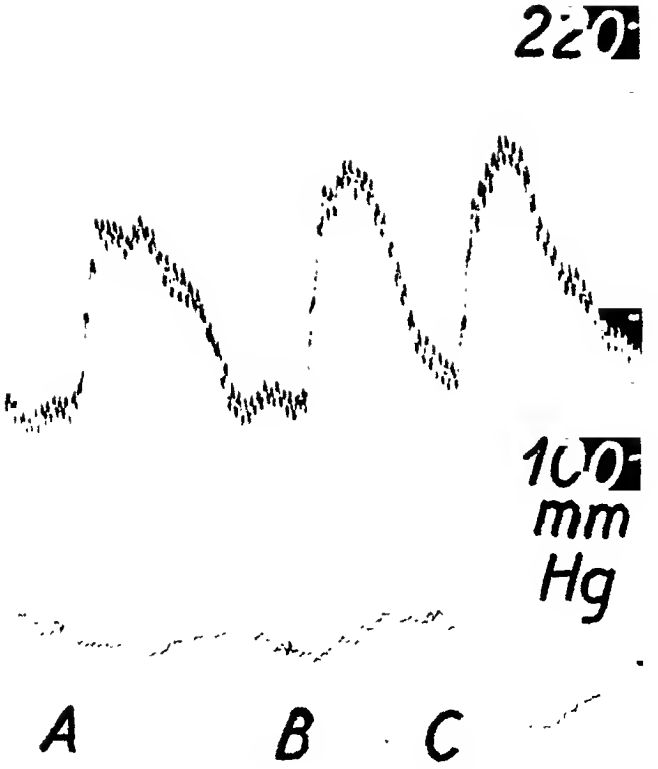

Fig. 28. Cat, blood pressure (upper curve), non-pregnant uterus in situ (lower curve). Both adrenals removed. Chloralose i. m.
After previous administration of Lergitin and atropine.
A. Extract from horse mesenteric arteries, corresponding to 1 g fresh tissue.
B. Extract from horse portal vein, corresponding to 1 g fresh tissue.
C. 3 μg adrenaline.
Time 10 secs.

Renal arteries. The extracts from renal arteries of the horse gave pressor effects and a relaxation of the non-pregnant uterus in situ, which was, however, not as pronounced as with equi-pressor amounts of adrenaline (Fig. 29). After dihydroergotamine the pressor effect of these extracts was reversed. When comparing the blood pressure effect of these extracts after ergotaminization with the effect of adrenaline it was found that equi-pressor amounts

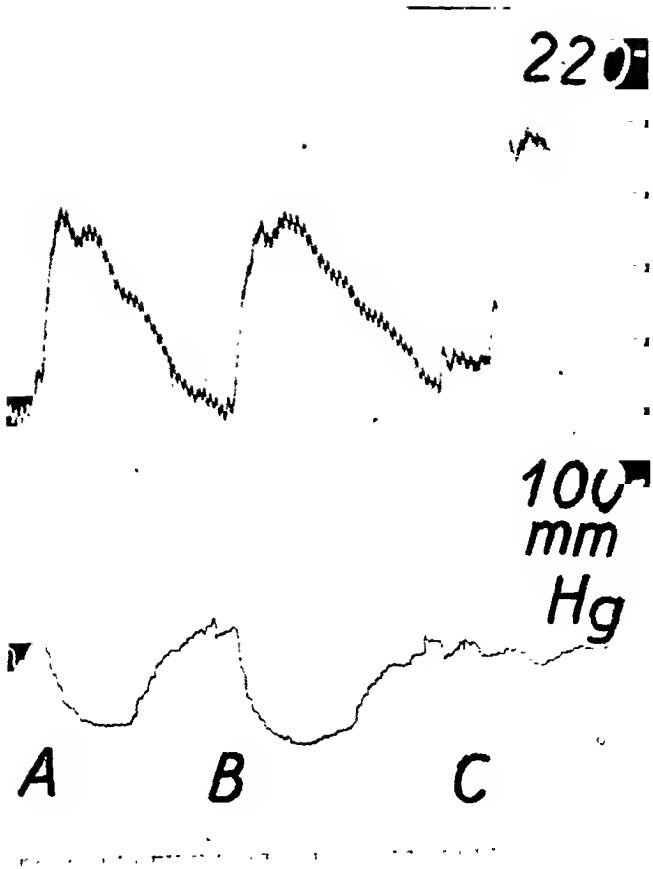

Fig. 29. Cat, blood pressure (upper curve), non-pregnant uterus in situ (lower curve). Both adrenals removed. Chloralose i. m.

After previous administration of Lergitin and atropine.

A. Extract from horse renal artery, corresponding to 1 g fresh tissue.
B. 4 μg adrenaline.
C. 3 μg l-nor-adrenaline.

Time 10 secs.

did not give exactly the same effect (Fig. 27, A, D). When testing the extract with SHAW's reaction there was a distinct increase in colour in the alkali-treated sample, which, however, was not as great as in the case of pure adrenaline. The extract gave a slight green fluorescence which was, however, less pronounced than with a solution of adrenaline in equi-pressor amounts.

All these facts suggested that the extracts from renal arteries contained a mixture of an adrenaline-like and a nor-adrenaline-like substance.

The amount of pressor substanee corresponded to 4 μg of adrenaline per g fresh tissue.

Vena cava. The amount of pressor substance in the superior vena cava corresponded to 3 μg adrenaline per g. The inferior vena cava contained considerably less, corresponding approximately to 0.5 μg adrenaline per g fresh tissue. In both cases the active substance behaved like nor-adrenaline and not like adrenaline.

Jugular vein. Extracts from the external jugular vein contained very little pressor substance, corresponding approximately to 0.25 μg adrenaline per g fresh tissue. It behaved like nor-adrenaline.

Portal vein. Extracts from portal vein contained a pressor substance, behaving like nor-adrenaline. The amount corresponded to 3 μg adrenaline per g fresh tissue (Fig. 28, B).

The amounts of histamine and acetylcholine in these extracts from different vessels were also determined. The results are found in the table on page 96.

Summarizing table

Blood vessel	Pressor effect corresponding to μg l-adrenaline hydrochloride per g fresh tissue	Depressor and smooth muscle stimulating effect corresponding to	
		μg histamine dihydrochloride per g fresh tissue	μg acetylcholine chloride per g fresh tissue
Aorta, proximal portion (cattle) .	0.5	9	0.4
» , posterior thoracic portion (cattle)	2	14	0.8
» , abdominal portion (cattle) .	1.25	11	0.7
» , (horse)	3	0.3	0.05
» , (dog)	2.5	0.9	traces
» , (pig)	2.5	0.9	»
» , (»intima layer», cattle) . .	0—0.25	2.5	0.05
» , (middle » ») . .	1	6.5	0.2
» , (outer » ») . .	1.5	10	0.5
» , (»intima layer», horse) . .	0	0	0
» , (middle » ») . .	1.5	0.1	traces
» , (outer » ») . .	3	0.25	»
Coronary arteries (horse) . . .	3	0.4	»
Mesenteric arteries » . . .	2.5	3	»
Femoral artery » . . .	0.75	0.7	0.1
Common carotid artery » . . .	0.75	0.8	traces
Renal artery » . . .	4	1.0	»
Vena cava superior » . . .	3	0.75	0.1
» » inferior » . . .	0.5	1.5	0.4
Jugular vein » . . .	0.25	0.8	traces
Portal » » . . .	3	1.25	»

(The figures given in this table are obtained as mean values from at least 4 different determinations.)

Chapter VIII

Discussion

Several authors have shown that stimulation of the sympathetic nerves to an organ caused a release into the blood stream of a substance which exerted sympathomimetic activity. Other authors have shown that extracts from different organs contain a sympathomimetic substance. In both cases it must be remembered that sympathetically innervated blood vessels are concerned. Some part of the sympathomimetic substance might thus be derived from the vessels.

The present investigation has revealed the fact that extracts (prepared according to the method described by EULER, 1946) from blood vessels, arteries as well as veins, contain a sympathomimetic substance. The extracts also contained substances with depressor and smooth muscle stimulating effects.

If a crude extract from *e. g.* cattle aorta was injected intravenously into the chloralosed cat the effect on blood pressure was a sharp fall followed by a secondary rise.

The identification of the depressor substances present in the extracts was considered necessary because if the "depressor phase" was separated from the crude extracts (using fullers' earth as an adsorbent, according to EULER, and then eluting the substances from this clay) it exerted an effect which in some respects resembled the effect of the crude extracts themselves, viz. an initial blood pressure fall followed by a secondary rise (see Fig. 6). Several facts pointed to the identity of this "depressor phase" with histamine. In a previous investigation (SCHMITERLÖW, 1948) it was found that the eluate from the fullers' earth which had been used to adsorb the depressor substance (or substances) from the crude extracts exerted the following effects:

1. A blood pressure fall (followed by a secondary rise) which could be abolished by a previous administration of an antihistamine drug.

2. A smooth muscle stimulating effect (test object: isolated guinea-pig's small intestine) which could also be completely blocked by small doses of an antihistamine drug.

3. A positive PAULY's reaction.

4. A "triple response" when applied to the human skin (LEWIS' reaction).

The fact that the "depressor phase" could in itself give rise to an effect which was rather similar to the effect of the crude extracts caused some confusion in the interpretation of the results.

When the crude extracts had been treated with fullers' earth they often gave a pure blood pressure rise. When testing these purified extracts on the isolated guinea-pig's small intestine they still, however, gave a contraction of the intestine. The removal of the depressor and smooth muscle stimulating substances was thus not complete but the purification procedure had led to a displacement of the balance between depressor and pressor activity with the result that only the pressor activity became visible.

Using two "sympathetic indicators", viz. the blood pressure and the non-pregnant cat's uterus in situ, the sympathomimetic effects of these purified extracts were further tested. Usually these extracts gave no relaxation of the uterus but sometimes a relaxation was observed. Furthermore, the pressor effect of these purified extracts was usually not reversed after ergotaminization of the test animals but sometimes a reversal was observed even when the effect before the administration of the ergot preparation was a pure rise in blood pressure. These divergences could be easily explained in the light of the finding that histamine was present also in the "purified" extracts. If histamine and nor-adrenaline were injected simultaneously the effect on the blood pressure could be a pure rise whereas the non-pregnant uterus showed a distinct relaxation (see Fig. 17). When injecting only nor-adrenaline the tone and rhythm of the uterus was not in-

fluenced. This showed that the relaxation of the non-pregnant uterus was due to adrenaline produced by the test animal itself and it is well known that histamine causes an increased output of adrenaline from the adrenals. The presence of histamine, not only in this "test" experiment but also in the purified (fuller-treated) extracts could explain the contradictory effects of the extracts as well as of pure nor-adrenaline (+ histamine) on the non-pregnant uterus. After ergotaminization the blood pressure raising effect of the extracts was diminished and if there was too much histamine left in the extract or if the test animal was extremely susceptible towards histamine there would be a new displacement of the balance between depressor and pressor activity, the latter now being — sometimes — overwhelmed by the former with the result that the pure pressor effect was seemingly reversed by ergotamine.

When testing the crude extracts on the isolated guinea-pig's small intestine it was observed that the contraction of the intestine could not be completely blocked by the previous addition of small doses of an anti-histamine drug to the suspension fluid even if equi-active amounts of histamine were completely counteracted. A small contraction still remained. In view of this fact it seemed necessary to investigate further the nature of the "depressor phase".

The isolated guinea-pig's small intestine was used as test object. The possibility of using an antihistamine drug ("Lergitin") as a sort of "specific denominator" for histamine was investigated. It was found that the effect of histamine was blocked by doses of Lergitin which were distinctly less than those necessary to block the effect of other spasmogenic substances. The specific (histamine antagonizing): non-specific (antispasmodic) dose ratio was determined. The same investigation was performed in regard to the "specific" anti-acetylcholine effect of atropine and also here it was found that the spasmogenic effect of acetylcholine was much more easily blocked by atropine than was *e. g.* histamine.

After adding a "specific" — only histamine antagonizing — amount of Lergitin to the intestinal bath the spasmogenic effect of the crude extracts from cattle aorta was not quite abolished even if the amount of Lergitin was increased about five times (to secure that all the present histamine was "covered" by the antihistamine drug). This remaining contraction could, however, be totally abolished by small "specific" doses of atropine. On the other hand, if specific doses of atropine were first added to the intestinal bath the spasmogenic effect of the crude extracts was somewhat diminished and this remaining contraction could be totally abolished by adding a "specific" amount of Lergitin. Using this alternate procedure the amounts of histamine and acetylcholine could be determined. The objection might be raised that these crude extracts also contained a sympathomimetic substance which could, being a sort of physiological "antihistamine" and "antiacetylcholine drug", counteract the effect of these smooth muscle stimulating substances. This objection could, however, be overcome. The sympathomimetic substance bchaved like noradrenaline (see later) and if the effect of this compound was tested on the spasmogenic action of histamine and acetylcholine it could be determined to what extent the presence of a certain amount of this sympathomimetic substance would counteract the effects of histamine and acetylcholine, and the figures then corrected.

In the light of the facts that the "depressor phase" consisted of histamine and acetylcholine and that even "invisible" (— as judged from blood pressure recordings) amounts of histamine may cause a disturbing outflow of adrenaline the further analysis of the "pressor phase" was carried out on adrenalectomized cats. In order to reduce or, if possible, abolish the effect of histamine and acetylcholine the test animals received Lergitin and atropine. If the "depressor phase" was not quite abolished by these drugs the crude extracts were treated with fullers' earth.

The nature and amount of the pressor substance (or substances) was then tested, the following tests being used:

1. The blood pressure of chloralosed, adrenalectomized cats with simultaneous observation of the heart rate.

2. The non-pregnant as well as pregnant uterus in situ of the adrenalectomized cat.

3. The blood pressure response after previous administration of dihydroergotamine or dibenamine.

4. The fluorescence test of GADDUM and SCHILD.

5. SHAW's colour reaction.

The first of these tests served only as a quantitative index of the amount of pressor substance. The other four tests served as a means of determining the nature of the pressor substance.

It was found that the pressor substance in extracts from cattle aorta quantitatively corresponded to 2 μg of adrenaline per g fresh tissue. Qualitatively this pressor substance did not, however, behave like adrenaline: it did not cause a relaxation of the non-pregnant uterus, its pressor effect was not reversed by ergotamine, it did not give the green fluorescence described for adrenaline by GADDUM and SCHILD and with SHAW's colour reaction the extracts did not give the specific colour increase typical of adrenaline. It might also be added that the effect on the pregnant cat's uterus in situ was different from that of adrenaline.

Aortic extracts from other species (horse, pig, dog) as well as extracts from different arteries and veins (horse) also contained a pressor substance, the quantitative estimation of which was performed and which did not behave like adrenaline except in the case of renal artery extract (horse) where an adrenaline-like action was observed. (This fact could be explained in two ways. 1. Adrenaline might be produced together with nor-adrenaline in this case. 2. Chromaffine cell groups have been observed in the renal hilus of the horse (ELLENBERGER-BAUM, Handbuch der vergleichenden Anatomie der Haustiere) and the presence of an adrenaline-like substance in extracts from the renal arteries might be due to an admixture from such cells.)

The pressor substance was sympathomimetic. It raised the blood pressure and accelerated the heart (cat), it caused a contraction of the pregnant cat's uterus in situ, it relaxed the isolated rabbit's intestine, it contracted the denervated nictitating membrane (cat), it caused a widening of the denervated pupil (cat)

and it exerted a positive inotropic and chronotropic effect on the frog's heart. Some of these criteria have been used throughout this investigation, some of them were performed in the beginning of this investigation but were not used for any other purpose than to secure the sympathomimetic nature of the pressor substance and are thus not mentioned in the experimental results.

When this fact had been established the question about the chemical nature of this sympathomimetic substance naturally arose. It could a priori be assumed that it was a substance closely related to adrenaline and presumably nor-adrenaline. The investigations of U. S. VON EULER have indeed pointed to this fact. In the present investigation the close resemblance between the sympathomimetic substance in blood vessel extracts and nor-adrenaline has been confirmed and even if no definite chemical proof of their identity has so far been produced there are no objections against the hypothesis that the active substance is really nor-adrenaline. Anyhow, it seems safer to state that the sympathomimetic substance acted like nor-adrenaline than to put an equality sign between the substance and nor-adrenaline.

The work of CANNON and LISSÁK (1939), previously cited, will now be discussed. They found that extracts from liver vessels contained a substance which behaved like adrenaline. Their findings have already been referred to (Chapter III) and some criticism may be advanced in regard to the interpretation of their results.

1. These liver vessel extracts produced a blood pressure rise which was enhanced by cocaine — a fact which also holds true for nor-adrenaline. After ergotoxine this pressor effect was reversed, suggesting adrenaline identity. But CANNON and LISSÁK point out that the blood pressure effect caused by these extracts before ergotoxine, was "mixed", *i. e.* in three cases out of four "the injection of an extract of the vessels from a normal liver caused, after a brief and minor initial drop in blood pressure, a high rise". As test animals they used cats with "adrenals tied off" but even if so, this initial blood pressure fall might be the explanation for this "reversal" of the pressor action after ergotoxine. It seems highly

probable that histamine was present in these extracts causing the initial blood pressure fall, and, as has been pointed out above, after the administration of ergot preparations there is a displacement of the balance between pressor and depressor activity. If the effect of the depressor substance is not counteracted it may overwhelm the effect of the pressor substance, the activity of which is already diminished by the ergotoxine. The result will be a "reversal".

2. Extracts from liver vessels also caused an extreme pupillar dilatation. As to the interpretation of this result some remarks are necessary (cf. EULER, 1946, d). Both adrenaline and nor-adrenaline cause a widening of the pupil, the effect of equi-pressor amounts of adrenaline being, however, definitely more potent. The difference in action is thus only quantitative. From the blood pressure recordings in CANNON and LISSÁK's paper it seems as if an amount of extract, corresponding to 1.5 g liver vessels, caused a blood pressure rise equal to that produced by 2 μg adrenaline. In their photographs of the sensitized cat's iris, however, the pupil dilating action of 2 g liver vessels seems to equal that of 0.5 μg adrenaline. The similarity between the action of liver vessel extracts and adrenaline on the iris may thus depend on the quantities used.

3. Extracts from liver vessels caused a contraction of the nictitating membrane. Both adrenaline and nor-adrenaline exert this excitatory effect, and the ratio of dose of dl-nor-adrenaline to an equi-active dose of l-adrenaline is, according to WEST (1947) only 1.25 which means that the nictitating membrane is not very suitable as a test object to differentiate adrenaline from nor-adrenaline.

4. The liver vessel extracts caused a contraction of the non-pregnant cat's uterus in situ and no relaxation. This contraction might have been due to the presence of histamine and as the adrenals were rendered inactive in the test animal no secondary relaxation occurred. This lack of relaxation seems to suggest that the active principle in the liver vessel extracts was n o t adrenaline.

5. The liver vessel extracts exerted a positive chronotropic and inotropic effect on the hypodymanic frog heart. Also on this test object there is only a quantitative difference in action between adrenaline and nor-adrenaline. From the recording from this experiment (Fig. 9 in CANNON and LISSÁK's paper) it seems evident that the concentration of the active principle of liver vessels must be high in order to reach the same effect as that of a very low adrenaline concentration. This would also suggest that the active principle was not adrenaline, since it is known (WEST, 1947) that the excitatory effect of adrenaline on frog's heart is considerably higher than that of nor-adrenaline.

From this discussion it might be infered that the findings of CANNON and LISSÁK do not prove that the active substance found in extracts from liver vessels was really adrenaline, it might just as well have been nor-adrenaline.

As was pointed out in Chapter III little was said about the action of extracts from different arteries, mentioned as extraction material in the same work of CANNON and LISSÁK (1939). It was, however, stated that they relaxed the non-pregnant uterus, a phenomenon which these authors found "puzzling and will require further investigation". In the present investigation no such relaxing effect of extracts from different arteries was found except in the case of renal artery extract.

Some remarks will also be made concerning the work of BACQ and FISCHER (1947). Preparing extracts according to EULER's method from human coronary arteries they found that of the three prepared extracts one contained a sympathomimetic substance which behaved like adrenaline and not like nor-adrenaline, and in the other two extracts they did not find any sympathomimetic substance at all. The fact that they found an adrenaline-like substance in only one of the three extracts and then in an amount corresponding only to 0.35 μg adrenaline per g fresh tissue, might be due to the circumstance that human material cannot, as a rule, be collected as soon after death as in the case of material from horse, cattle etc. BACQ and FISCHER used the denervated nictitating membrane and the non-pregnant uterus

in situ of the cat as sympathomimetic indicators. In one of the recordings in their paper it is seen that a purified extract of human coronary arteries and nerves caused a relaxation of the uterus. It is not stated, however, if the cat used in this experiment was adrenalectomized, it is only pointed out that "dans certaines préparations, nous avons aussi ligaturé les deux capsules surrénales".

In the beginning of the present investigation only dl-nor-adrenaline was available and the comparisons between the sympathomimetic substance and nor-adrenaline had thus to be performed with this racemic substance. Later on l-nor-adrenaline could be obtained. As l-adrenaline is the naturally occurring form of adrenaline it might be argued that if the sympathomimetic substance is nor-adrenaline it is highly probable that it corresponds to l-nor-adrenaline (cf. WEST, 1947).

Nor-adrenaline is by no means a "purely excitatory" substance — it exerts inhibitory actions as well as excitatory. This fact has been pointed out by several authors. It is true that it did not exert any inhibitory action on the non-pregnant uterus in situ but on the other hand it gave a distinct relaxation of the isolated rabbit's intestine.

BACQ and FISCHER (1947) have already pointed out that the active sympathomimetic substance in extracts made according to EULER could not be regarded as an artifact caused by the extraction procedure itself. The present investigation has also shown that the nature of l-adrenaline, dl-nor-adrenaline and l-nor-adrenaline was not changed by the extraction procedure even in the presence of organic tissue (containing no sympathomimetic substance itself — placenta). A slight loss in activity was observed but no change in the mode of action of these three compounds.

If the pressor substance is sympathomimetic it seems reasonable to argue that its presence in the extracts has a close relation to the innervation of the extracted tissue. EULER (1946, d) found a sympathomimetic substance with the properties of nor-adre-

naline in adrenergic nerves and had previously (1945) found that there was no such active substance in the nerve-free placenta.

It was found in the present investigation that different parts of the cattle aorta contained different amounts of the sympathomimetic substance and it was then found that this difference coincided with a difference in innervation — the portion of the aorta which at histological examination (Professor A. PALMGREN) was found to contain most nerve stems also contained most sympathomimetic substance — and also most histamine and acetylcholine. If the intima layer was separated from the rest of the aortic wall (which was especially simple in the case of horse aorta) and the rest of the aortic wall was then divided in two layers it was found that extracts from these three separate layers contained different amounts of sympathomimetic substance, histamine and acetylcholine. The intima layer contained practically no sympathomimetic substance, the middle layer some and the outer layer most.

These findings in correlation with the findings of EULER that adrenergic nerves themselves contained a sympathomimetic substance and that the nerve-free placenta contained no such substance strongly suggested that the presence of sympathomimetic substance in the blood vessel walls is due to the presence of sympathetic innervation of the vessels.

EULER (1948, a) estimated the amounts of sympathomimetic substance, histamine and acetylcholine in different nerves. He found that the postganglionic adrenergic splenic nerve (cattle) contained an equivalent of 10—25 μg dl-nor-adrenaline, 60—120 μg histamine and 0.2—0.5 μg acetylcholine per g fresh tissue. The proportion between these three "ergones" corresponds fairly well to the proportion found in aortic wall of cattle, the amount of acetylcholine being, however, higher in the aortic extract. It is surprising to find that the amount of sympathomimetic substance found in extracts from postganglionic nerves was only about 10 times higher than the value found in extracts from the aortic wall. As it is impossible to believe that $^1/_{10}$ of the aortic tissue would consist of nervous structures the following hypothesis might be evolved. In the

ganglionic cell (the pericaryon) the sympathomimetic substance is built up either as such or in the form of a precursor. This substance is then transported peripherally in the axoplasm. If it is produced as the active substance itself it must be supposed that it is in some way accumulated towards the nerve endings as the concentration of the active substance in the extracted tissue (be it aortic wall or spleen, EULER, 1946, a) is much higher, calculated per weight unit of nervous tissue, than in postganglionic adrenergic nerves. This accumulation is difficult to explain and it is easier to believe that the active substance is produced in the form of a precursor. Some of this precursor but only a small part is changed to the active substance (nor-adrenaline) already in the postganglionic nerves whereas at the nerve endings this precursor is changed to become the active substance, extractable from the tissue.

In the light of the experimental facts of recent years the hypothesis of CANNON and ROSENBLUETH about sympathin E and sympathin I seems unsatisfactory. According to their opinion a common mediator (M), presumably adrenaline, should be liberated at sympathetic nerve endings and would then combine in some way or other with an excitatory or inhibitory substance within the reacting cells. In view of the findings of EULER that a sympathomimetic substance of nor-adrenaline character is present in adrenergic nerves themselves and the fact that the same sympathomimetic substance is present in effector organ, viz. the smooth muscles of the blood vessel walls the intervention of intracellular substances seems unnecessary. If the sympathomimetic substance in adrenergic nerves and in blood vessels is nor-adrenaline it seems unlikely that this nor-adrenaline should suddenly change over to adrenaline when released at the nerve endings and then change back to nor-adrenaline again in the innervated tissue. It is also worth mentioning that if adrenaline or nor-adrenaline was added to minced placenta no change in the nature of these two substances occurred although smooth muscle cells are present in the placenta vessels. The fact may also be pointed out

that after denervation of the liver vessels no sympathomimetic substance was found in extracts from these vessels (CANNON and LISSÁK, 1939). Also in the case of the spleen a great reduction of the content of sympathomimetic substance was found after degeneration of the main portion of the postganglionic periarterial splenic nerves (EULER, 1946, d).

The suggestion of EULER (1948, b) to use the distinctions sympathin N and sympathin A, signifying mediators with the properties of nor-adrenaline and adrenaline respectively seems to satisfy the experimental results concerning the nature of the sympathetic transmitter.

The sympathomimetic substance found in extracts from blood vessels is obviously sympathin N except in the case of extracts of renal artery from the horse where an admixture of sympathin A was found.

Summary

1. Using the extraction method described by EULER (1946) extracts have been made from different blood vessels, including aorta, coronary, mesenteric, femoral, common carotid, and renal arteries, vena cava, jugular and portal veins from different species.

2. These extracts contained depressor and pressor substances.

3. Tests performed on isolated guinea-pig's small intestine as well as blood pressure recording on the cat showed that the depressor activity was due to the presence of histamine and acetylcholine. Using an antihistamine drug and atropine a method has been elaborated by means of which it was possible to assay the amounts of histamine and acetylcholine.

4. The presence of histamine in the extracts makes the biological identification of the pressor principle difficult as even traces of histamine, though masked in its action on the blood pressure by the pressor substance may cause an increased output of adrenaline from the adrenals of the test animal itself. In order to avoid this error the biological analysis of the pressor substance was performed on adrenalectomized test animals (cats) and to counteract the actions of histamine and acetylcholine an antihistamine drug and atropine were administered to the test animals.

5. The pressor activity was found to be due to the presence of a sympathomimetic substance.

6. "Test" experiments were carried out to control the extraction procedure and it was found that the extraction method did not change the nature of the sympathomimetic substances adrenaline and nor-adrenaline.

7. Using biological as well as physical and chemical tests it was found that this sympathomimetic substance behaved like noradrenaline and not like adrenaline, except for the renal artery extracts from the horse, which obviously also contained a substance acting like adrenaline.

8. A correlation was found to exist between the amount of pressor (and depressor) substance and nervous structures in the extracted tissue.

9. A hypothesis is evolved in order to explain the puzzling fact that the amount of sympathomimetic substance in the aortic wall is very much greater than could be calculated from the figures given by EULER in extracts from adrenergic nerves (calculated per weight unit of nervous tissue).

10. The nature of the sympathomimetic substance is discussed in relation to the sympathin E and I of CANNON and ROSENBLUETH and sympathin N and A of EULER.

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